

Public Money for University Research

UNIVERSITIES are notoriously proud of their independence, both individually and collectively, which is why it is such a great surprise that the Science Research Council has been able to keep its friends in the universities while persuading them to change their ways quite fundamentally. Within three years, the council has established the principle that the pattern of university research should be determined not by the statistical aggregation of the inclinations of university teachers but by a more deliberate process of planning in the course of which the council itself has assumed responsibility for picking out desirable directions for research. At the beginning, to be sure, a great many academics were up in arms, saying that academic freedom would be in jeopardy if the Science Research Council chose to head people off certain lines of enquiry or even to concentrate desirable work in selected universities. Some mathematicians are still complaining (see *Nature*, **233**, 225; 1971), but that protest has all the appearance of a rearguard action. Most university departments in Britain are resigned and even glad of the Science Research Council's initiative.

This is why one of the biggest dangers now facing the Science Research Council is that of complacency. The most recent annual report (see page 298) is a vigorous document, but there is a mild suspicion that the council may be more ready than is strictly necessary to compromise with unpleasant truths.

The chief of these is money. In the past year, it has been made known by the present government that the funds available for university research would grow less quickly than the numbers of students in higher education and therefore, by definition, that the proportion of university teachers able to recruit financial support from the research councils would diminish. This simple truth was spelled out clearly enough at the conference organized by the Science Research Council in Manchester last January. To everybody's surprise, the universities have raised no audible protest. By now the policy is accepted, and it is entirely appropriate that the Science Research Council, the principal source of grants for university research, should have decided that the time has now come to end the automatic but cumbersome procedure by means of which funds are transferred from the council to the University Grants Committee at the end of each university quinquennium (the next comes in July 1972) so as to keep in being research projects begun by the research councils. Under the new system, discrimination will be easier. Universities will be under less pressure to institutionalize research groups. To the extent that these effects are consonant with what the Science Research Council has been looking for, it is clear that the grand design can be implemented even more efficiently in the years ahead.

It is therefore proper to ask whether the assumptions on which the new policy is based are as appropriate as everybody supposes. One of the Science Research Council's objectives is to create a greater sense of liveliness in university research and so far, with some success, it has sought to do this by providing not merely research grants but also facilities—laboratories for high energy

physics or for astronomy, contributions to organizations such as CERN and even arrangements for making sure that universities have access to computing machinery. Yet the Science Research Council remains only one of the four organizations responsible for channelling money towards science departments in universities. The three other research councils are smaller in aggregate but just as influential in their somewhat narrower fields. It is all very well for the Science Research Council to boast in its most recent report that all four of them have been able to put their names to a document in which four separate statements on pollution appear within the same cover, but that is a far cry from the coordinated policy on research which British universities now require. Why, after all, should it be the Science Research Council that makes grants for research on high pressure physics but the Natural Environment Research Council that gives out money for closely related topics in geology? Why should it be that the Science Research Council administers central facilities for high energy physics but that the Natural Environment Research Council looks after oceanography (with all its sea-going vessels)?

Another problem concerns the scale on which university research should be carried out. In past decades, the conduct of university research in Britain has been bedevilled by self-justifying prophecies about the capabilities of university departments to look after their own affairs. Large items of equipment have frequently been placed on extramural territory not merely because sharing among several universities is intended but also because it has been held that universities are unable to manage large installations (a notion easily disproved by the radio astronomy observatories which, by historical accidents, are in the pockets of university departments). As the policy of selectivity and concentration succeeds, however, there will be more and more arguments in favour of letting British universities do what American universities have done for the past three decades—to take managerial responsibility for large laboratory installations. When that time comes, the budget of the Science Research Council and its smaller associates should increasingly be spent within universities. That tendency is not merely something to be stomached—it is itself desirable.

A third class of problems stems from graduate education in British universities. For years now, the Science Research Council has been saying that it wishes to encourage a different pattern of postgraduate teaching. Courses should be more relevant to the needs of industry, and students should be more alert to them, but at the same time there is a need somehow to broaden the base of postgraduate teaching so that graduates emerge as more flexible people. So far, these declarations have gone little further than wishful thinking. Yet the fact remains that British graduate education, good though it may be as an intellectual training for enquiring minds, is unsuited to the needs which taxpayers hope to meet by keeping universities in the state of grace to which, quite properly, they say they are entitled. What the Science Research Council should now do is to back up promises with performance.



Publish and be Damned a Second Time

Now that Mr Herman Kahn has made it respectable to think the unthinkable, it may be appropriate to ask what virtues still attach to the convention that reports of original scientific research should not be published more than once. Most scientific journals, *Nature* included, take a stern view of breaches of this convention and most scientists seem first of all to approve of the convention and then to do their best to live by it. The arguments for a rigid rule against multiple publication are of course familiar. The literature is full enough already, and if multiple publication became common practice, the speed with which libraries are forced to throw up their hands in despair would be enormously increased. But multiple publication can also cause bibliographical chaos. The problems of knowing whether two apparently similar titles by the same authors refer to substantially the same original article would turn every working scientist into a textual analyst. At the same time, it is only fair to recognize that there have grown up practices which consist of variations of the strict convention that those who publish the same article twice are guilty of dishonourable conduct. By now, for example, preliminary communications are widely recognized to be useful ways of making public important discoveries, but there are good reasons for complaint if the fuller version of the discoverer's report does not eventually materialize. More reprehensible, there has been a tendency in the past few years for reports of original research, once published, to pop up again in closely related forms as contributions to symposia, international conferences and other occasions on which professional scientists have been persuaded to travel. The equation between the seminar paper and the airline ticket is now uncomfortably precise.

In all these circumstances, it would of course be over-zealous of a journal such as *Nature* to follow too rigid a policy, but there are some circumstances in which multiple publication is not merely an offence against accepted conventions but an assault on ordinary standards of decent behaviour. Earlier this year, there appeared in *Nature Physical Science* an article of some importance which was described by the principal author as "an extremely important breakthrough into the new field of . . .". With all despatch, the article was sent to a referee and published in the usual way. Only afterwards did it become known that the same article had been submitted to two other journals. By all accounts, it seems that the article was rejected by one journal but published, in a format substantially the same as that which appeared in *Nature*, by the second. Since then, the hapless authors have been assailed by several editors and the senior author has actually written to say "We were all very excited at the time and I would like you to think that my apparent breakdown in publication ethics was the result of over-enthusiasm." With some regret, it has been decided not to publish the names of those concerned in this shabby business, but to scrutinize with immense care their claims to originality for other publications. In the long run, however, a public register of such offenders may turn out to be the best method of keeping authors on a straight and narrow path. No doubt such a record would have some of the attractions of a gossip column as well.

Another issue involving a form of alternative publication is much harder to resolve. In the past two decades, newspapers have become increasingly enterprising sources of scientific news. In the long run, of course, this is of public benefit, but there have been times when the editors of journals have been embarrassed to discover that the essence of some cherished article has been given to the daily press in such detail that full publication seems a mere formality. Thus there has grown up a running battle between some journals, with *Physical Review Letters* at their head, and those newspapers and magazines which have such a flair for seizing on important discoveries that they are, almost by definition, the best newspapers. It is only fair to say that the distinction between proper and improper practices is extremely hard to make in this connexion. Certainly there can be no rigid rule. It is also unreasonable to expect that the authors of an important discovery should keep this strictly to themselves for months on end while waiting for the solemn processes of formal publication to be completed. Not merely is this unnecessary but impractical as well, now that journalists habitually turn up at scientific meetings. And it would, of course, be altogether too toffee-nosed to ask that news of scientific discoveries should be kept even from the audiences at scientific meetings until the mills of the publication process have begun to grind. So it seems to follow that journals, *Nature* included, can ask authors to refrain from telling newspapers their good news only if the process of publication is reasonably swift. What matters is that the relationship between an author and the editors of the journal in which he seeks to publish should be open and courteous. And even then, there are bound to be occasions when news leaks out. In short, although journals are right to ask that their contributors will not call a press conference whenever a manuscript is accepted for publication, their best insurance against being scooped by the newspapers is to publish quickly.

100 Years Ago



THE First Commissioner of Works and Public Buildings announces that he intends again to distribute this autumn, among the working classes and the poor inhabitants of London, the surplus bedding-out plants in Battersea, Hyde, the Regent's, and Victoria Parks, and in the Royal Gardens, Kew. If the clergy, school committees, and others interested, will make application to the superintendents of the parks nearest to their respective parishes, or to the director of the Royal Gardens, Kew, in the cases of persons residing in that neighbourhood, they will receive early intimation of the number of plants that can be allotted to each applicant, and of the time and manner of their distribution.

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OLD WORLD

EUROPEAN LABORATORY

ESRIN in Trouble

A PROPOSAL to close down the European Space Research Institute (ESRIN) is widely regarded as a setback to the cause of European collaboration. The laboratory is one of several established by the European Space Research Organization. It was originally intended as a centre for fundamental research relevant to ESRO's activities in space, and has been particularly concerned with plasma physics. If a resolution introduced at the ESRO council meeting in July should be adopted in November, the laboratory will be run down in 1972 and 1973.

The ESRIN laboratory is at Frascati, south of Rome. It has a permanent staff of about 70 and an annual budget of \$2 million. In the past months, the institute has attracted a steady stream of visitors who add about a dozen to the professional complement. The director of the institute, Dr Nicola D'Angelo, succeeded Dr H. L. Jordan in 1970. He is thought to have received many expressions of support in the past few weeks, especially from university scientists, but there are fears that these may not be sufficient to distract ESRO from its present preoccupation with applications satellites.

Apart from purely budgetary considerations, it is possible that ESRIN has been threatened because the work it does might just as well be done in universities, although one of those involved says that the institute was originally set up to tackle problems beyond the reach of university departments, and much of its equipment reflects this intention. Evidently the peripheral role of ESRIN within ESRO has weakened its claim on public funds, but its supporters claim that it is one of the few examples of an international laboratory which has been an entire success.

Present and former members of ESRO complain that it will be a great waste if the laboratory is closed down just when it has been built up to full strength. Younger scientists who have recently left apparently secure positions in their own countries to work at an international laboratory are likely to be left with a great sense of frustration. Investment in ESRIN so far amounts to \$10 million. The annual running cost is 2.5 per cent of ESRO's total budget of \$80 million. Although it may be held that ESRIN was not strictly necessary, closing it down is certain to be widely regarded as a setback.

DETERGENTS

Phosphates to Stay?

COMFORTING remarks about the effects of detergents on the environment have become a feature of the annual reports of Procter and Gamble Ltd. In a special section in the report for 1970-71, the company says, for example, that the work carried out in Procter and Gamble's laboratories on enzyme washing powders shows that they are "safe for consumer use" and it quotes the British Government's Standing Technical Committee on Synthetic Detergents as support for its contention that enzyme detergents do not pose any problems in rivers or in sewage works (*Twelfth Progress Report of the Standing Technical Committee on Synthetic Detergents*, HMSO, 1971; £0.22½).

As far as phosphate residues are concerned, the Procter and Gamble report again quotes the Standing Technical Committee and says that it is wrong to assume that the eutrophication problems experienced in the United States are applicable in Britain. In the United States, much more sewage is discharged into lakes where there is little water movement and the largest single cause of eutrophication is thought to be compounds such as sodium tripolyphosphate which are included in detergents as water softeners and emulsifying agents. A recommendation by a United States congressional committee in April 1970 sent detergent manufacturers hurrying off to develop alternatives, but three government agencies last week declared in favour of phosphates because they seem to be less of a hazard than some of the substitutes, such as sodium nitrilotriacetate, about which there are still unresolved doubts on health grounds (*Nature*, 233, 229; 1971).

According to the Water Pollution Research Laboratory at Stevenage, only 46 per cent of the phosphate content of sewage in Britain is in any case attributable to detergents, the remainder arising from human excreta and from industrial effluents (chiefly from metal finishing processes). Only about 70 per cent of the phosphate content of detergents could be replaced without seriously impairing the detergent function, so the advantage to be gained from the introduction of other compounds—whose properties are relatively unknown—would only be a decrease of about 30 per cent in the proportion of phosphate in sewage. It looks as though phosphate detergents are here to stay, at any rate until the alternatives have been proved to be safer.

The report of the Standing Technical Committee also says that even if phosphate-free detergents were manufactured in Britain, it is unlikely that the growth of algae would be very much inhibited.

INTELLIGENCE

Who Watches Whom?

from a Special Correspondent

THE first international conference on Communication with Extraterrestrial Intelligence on this planet was held at the Byurakan Observatory in Soviet Armenia from September 5 to 11. A joint project of the US and USSR Academies of Sciences, the conference was attended by fifty-six official delegates with one representative from each of Czechoslovakia, Hungary and the United Kingdom. There were present not only astronomers and communications experts, but biologists, historians, anthropologists, linguists and others, including two Nobel laureates, Charles Townes and Francis Crick. The subject, once treated as a bemusing speculation, is increasingly respectable.

At the symposium, much attention was given to the abundance of technical civilizations in space. Some old uncertainties were disposed of. In particular, the neurophysiologists painted a clear picture showing that one should expect everywhere the development of a complex central nervous system leading to an intelligent brain. The historians showed that this in turn leads to a highly systematized and organized society which inevitably develops technology. Ignorance in key areas, however, haunted the discussion. Although the first steps in the evolution of complex organic molecules from the simple molecules of the primitive planetary atmospheres have now been traced, these experiments have not come close to producing the key molecules of contemporary life such as DNA, and have not demonstrated that the development of such molecules is inevitable. The uncertainty prevents the transfer of the origins of life from the realm of miracles to that of statistics, to paraphrase Philip Morrison. We fall back on subjective probabilities of great uncertainty.

The abundance of detectable civilizations should be proportional to their longevity as power emitting entities. Fears have grown that civilizations only slightly ahead of those on this Earth will be so good at conserving energy that there is none wasted for us to detect. Indeed, it may be possible to detect only those that want to help us, and even those may be few; as Carl Sagan put it, they may have little interest in conversing with a species as antique, in their view, as we are. Compounding the probabilities, both statistical and subjective, the feeling developed that it was probably thousands of light years to the nearest detectable civilization.

It was noted that we might first detect other civilizations through evidence of astro-engineering activities such as the

construction of devices to capture all the energy of a central star, this energy eventually appearing as thermal pollution in the far infrared.

But it was still quite generally agreed that the most likely route to detection of and communication with other civilizations is through electromagnetic radiation particularly at radio frequencies, where some existing systems could detect themselves across half the galaxy. There is still a search for syllogisms which would lead to optimum frequencies and places to search. Surprisingly, what had been a simple situation has become complicated. Where once there was one special radio wavelength on which to listen for other creatures, the 21-cm wavelength of hydrogen, there is now a multitude, including spectral lines of water, the stuff of life, more intense than any hydrogen line. There is no clear choice among them. Once there was a microwave frequency where the cost of transmission was minimal, with the cost increasing at lower frequencies due to cosmic noise, and at higher frequencies due to quantum noise. Now the existence of the universal 3° black body radiation has added a new noise source which spreads the most economical frequency from 1,200 MHz to 60,000 MHz. To make things worse, it no longer even makes much sense to examine the nearest stars first. It has been shown that if there is a range in the luminosities of civilizations in which only a small percentage are much more luminous than the rest, then the ones most visible to us will not be the nearest but the intrinsically brightest. These may lie in any direction, and so there is no guidance as to where to look. Such a situation is not unprecedented; the radio galaxies exhibit just this behaviour.

Despite these new uncertainties, the much discussed conclusion that success would lead to great scientific and social benefits, and the striking potentialities of the existing instruments, produced a remarkably strong consensus that major efforts should be made to search for extraterrestrial intelligent life. It was recommended that existing instruments make the valuable and promising searches they can make. Among other recommendations, it was also suggested that studies should be made of the design of giant instruments for searches: a radio telescope 1 km or larger, a 100 metre millimetre wave telescope, a thirty metre submillimetre wave telescope and a ten metre infrared telescope. The first of these, which has many other uses, could cost billions of dollars, a figure thought, after all, not out of keeping with the importance of the subject, and consistent with the sums spent on space and nuclear research. The Soviet workers at Gorky,

under V. S. Troitsky, have in fact recently initiated a search for signals, so far testing a dozen stars, and watching for pulsed emissions from anywhere in the sky; the work continues.

The conference set up a committee to organize future conferences and meetings to coordinate research, if desirable. So far, such organizational activities and actual research have been confined entirely to the United States and the Soviet Union for no profound or good reason. It was expected that participation in the discussion and research in extraterrestrial intelligent life will spread elsewhere.

HUMAN ECOLOGY

Hopes Balance Fears

OPTIMISTS and pessimists were evenly matched at the annual symposium of the Eugenics Society in London last week. The subject was the now familiar population and pollution and Dr Eliot Slater of the Institute of Psychiatry, University of London, began with a warning that population growth, which meant that "a man does his neighbour more harm than good just by staying alive", was forcing society to adopt new standards of behaviour which were to him distasteful. Population pressure was undermining people's natural benevolence. The maintenance of social order requires more and more state intervention, with the result that human identity will be "increasingly translated into a set of digits on a computer". More crowding means more arousal—then drugs and even greater dangers, rage, despair and organized violence. The nuclear family has already been reduced and the pace of social change is such that "we are like a man racing down a mountainside whose legs cannot keep up with his centre of gravity". Does the encouragement of suicide follow the encouragement of abortion? Is it possible to avoid disaster if we regard man as a measure of all things?

The anthropologists took a longer view. People, even primitive people, have always damaged the environment. They are only worried about it now because they can no longer escape the results. The moors of Yorkshire and Wales, like the deserts of North Africa, are monuments to previous occasions on which people have been able to upset the delicate ecological balance. Pollution is not new, only the perception of it.

The environmentalists tended to the view that pollution is a problem in public administration. The urgent need is for collaboration at the level of local, national and international government. The herrings are disappearing from the

North Sea, but DDT is probably not the explanation. So far as this symposium is concerned, the moral seems to be that the fight against pollution must and should begin at home.

The geneticists were more worried by chemicals than by radiation. Dr Mary Lyon, Girton College, Cambridge, argued that the radiation from nuclear fall-out is likely to be less mutagenic than the medical use of X-rays. Dr M. S. Legator of the United States Food and Drug Administration, on the other hand, took the view that respectable investigations of the mutagenic effects of certain chemicals had only just begun.

The sociologists opened with a new warning that it is affluence and not technology or over-population that spells pollution. In the maddening manner of their discipline, they argued that research tends to be concentrated not on the important problems but on those which can be solved, however trivially. To them, to be practical, zero or even negative population growth would be a worthwhile goal.

The capstone of the symposium was the Galton lecture by Dr C. L. Carter, an optimist. The new eugenics, he implied, is the amalgam of social work and genetics which makes it possible to avoid needless misery while allowing people freedom. Dr Carter argued that once family planning is effective the more intelligent parents will have the larger families, resulting in a higher average IQ. He looked for an even more rapid adaption in the future of "genetic endowment to fit society".

PATENTS

Inventions Incorporated

PLANS for the establishment of a European patent office are finally taking shape and the first European patents will probably be granted in 1973. The intergovernmental conference that has been sitting since 1969 has recently published a draft agreement and it is hoped that final recommendations will be made by June 1973.

The notion of a European patent was first put forward in 1955 by the Council of Europe, which agreed on a system of classification of patents that was eventually accepted in 1968. In 1962, the European Economic Community set up a working party to prepare a draft European patent but the plan was shelved until 1969, when the present conference of nineteen European countries was set up.

The European patent is being welcomed by the British Patent Office and Mr J. D. Fergusson, assistant comptroller, estimated this week that the setting up of a European patent office will decrease the number of annual

applications from 60,000 to 155,000 or 20,000. He added, however, that the reported date of achieving the European patent was highly optimistic and that 1976 would be more realistic. The decrease in the number of applications to the British office would be reflected by a decrease in staff but it would not lead to redundancy as the European office would need qualified people. The site of the European office has not been decided although Mr Fergusson said that it must be within an EEC country.

The advantages of a European patents office are that it will avoid duplication of applications and work. Ideally, national patent offices should cease to exist when the European office is working, but Mr Fergusson said that it was not planned to close the British office and that a substantial number of applications would still be received as the cost of obtaining a European patent would be high. Most of the overseas applications, now about 60 per cent of the total, would go to the European office but a trickle of applications from the United States would still be received. The British Patent Office is planning to carry out a market research survey in 1972 to see how many of the British applications will be channelled through the European office.

The standards to be set by the European office are more stringent than those now in force in Britain and most European countries and comparable to those of West Germany. Agreement has already been reached by the nineteen countries presently negotiating that if the European office finds a patent invalid then it will also be declared invalid in the member countries. If an application is made simultaneously to the British and European offices, Mr Fergusson said that the British application would be suppressed until the result of the European patent is known.

The logical step to take after setting up a European office would be to obtain world-wide standardization of patents. Mr Fergusson said that this was not feasible because of the different concepts—now standardized in Europe—of the essential ingredients of a patentable idea. It seems, however, that this would be a laudable aim and the Patent Cooperation Treaty set up last year (see *Nature*, **226**, 888; 1971) goes a long way towards achieving it. This treaty eases the work on patent offices by sharing the exploratory work, but applications have still to be made to individual countries to register patents.

COMPUTERS

Profit the ERA Way

With declining sales and profits rapidly turning into losses in the computer service industry, it is surprising to find

the Electrical Research Association launching a new company, Electra Computer Services. But this is a logical development of ERA's activity in the computer field over the past two years, and points the way for future profitable developments in the industry. The organization of the ERA is more like that of comparable American research institutions than of British research associations—more than 90 per cent of its activities are carried out under contract without the aid of government funds. There is no government support for the computer unit, which was established two years ago, chiefly for the benefit of members of the ERA.

Why has the ERA been successful when other computer service companies are falling by the wayside every week? The association says that it provides a personal service. The managing director of the new company is Mr Paul Addison who has headed the Association's computer activities since they started. He emphasized that customers are not really concerned with how a computer bureau solves their problems but only the accuracy of the solution. Whereas most bureaux fall into one of two categories: providing solutions to scientific

and technical problems by the flexible use of their hardware, or selling off the peg packages to solve standard problems such as payroll calculations—the bureaux of the future must provide a complete data processing service to be credible. It is the aim of the ERA to provide such a service with Electra, solving unique problems or providing standard services as required, so that the customer has the same sort of close contact and versatility which he could expect from his own in-house computer.

The association claims that its customers have been persuaded by experience that it is often better to buy computer time rather than a machine. In one case, for example, it says that a customer was able to reduce his annual expenditure from £70,000 to £15,000 by forsaking his machine for the association's service. The association also claims its reputation with industry now allows it to give potential clients a feeling that the provision of computer services is not another way of robbing them of cash. For customers new to the use of electronic computers the ERA says that its services are a useful intermediate step to the purchase of a machine.

Appointments

MRS MARGARET THATCHER, Secretary of State for Education and Science, announced last week the appointment of new members to the Natural Environment Research Council, Medical Research Council, Computer Board for Universities and Research Councils and the Advisory Committee for Scientific and Technical Information.

Four new members are appointed to NERC: Professor P. Allen, University of Reading, Professor R. B. Clark, University of Newcastle upon Tyne, Professor K. M. Clayton, University of East Anglia, and Dr H. C. Pereira, East Mallory Research Station. From October 1, 1971, NERC will be composed of the new members together with Professor F. H. Stewart as chairman, Professor Sir Edward Bullard, Professor R. W. Edwards, Lord Howick of Glendale, Rear Admiral Sir Edmund Irving, Dr C. E. Lucas, Mr T. A. L. Paton, Professor F. W. Shotton and Sir Owen Wansbrough-Jones.

The Medical Research Council also has four new members: Professor D. A. K. Black, University of Manchester and Manchester Royal Infirmary, J. P. Bull, MRC Industrial Injuries and Burns Unit, Birmingham Accident Hospital, D. G. Davey, Imperial Chemical Industries, and Professor R. B. Welbourn, University of London, Royal Postgraduate Medical School and Hammersmith Hospital. The non-retiring members of the council are: His Grace the Duke of Northumberland (chairman), Professor Sir Richard Doll, L. Pavitt, Professor W. S. Peart, Professor D. A. Pond, Professor R. R. Porter, Professor T. Symington, Professor P. M. B. Walker, Professor R. E. O. Williams and Dr J. A. B. Gray.

The Advisory Committee for Scientific and Technical Information is increased to twelve members by the appointment of Professor G. Black, University of Manchester, Professor L. Maunder, University of Newcastle upon Tyne, and Dr R. Weck of the Welding Institute to the committee. The other members are: Dr L. Rotherham, chairman, Dr J. W. Barrett, Mr J. G. N. Brown, Dr G. M. Dyson, Mr. H. J. Habakkuk, Professor J. B. Jepson, Mr N. Mackenzie, Mr D. T. Richnell and Professor G. D. Sims.

Professor G. A. Barnard, University of Essex, and Professor J. C. Gunn, University of Glasgow, are appointed to the Computer Board for Universities and Research Councils. The other members of the board are: Professor D. J. Finney, chairman, Mr A. H. Duncan, Dr J. Howlett, Dr K. W. Morton, Professor D. J. Newell and Professor M. H. Rogers.

Gesture of Independence

THE Science Research Council has given the government a clear hint in its report for the year ending on March 31 (HMSO, £0.65) that the present review of arrangements for spending public money on scientific research should not be completed without full consultation. In particular, the council says that it "looks forward to the publication of the Dainton report and the opportunity to discuss its findings before any decisions are taken on the reorganization of civil science". The Dainton report, commissioned by the Council for Scientific Policy, was completed in May this year, and has since been regarded as a part of the material with which the Rothschild review should be provided.

The Science Research Council is also as open about its wishes as any official body might dare. The report says that the council reaffirms "its belief in the present role of the research councils and stresses the need for close collaboration and cooperation between all the bodies contributing to the support of civil science". It also asks for closer links between "those in industry and government who are concerned with the role of science in national life". Although the council can boast only of the somewhat lukewarm collaborative document on pollution research as evidence of the way in which the research councils might collaborate, it promises more studies of other common interests.

In its own defence, the Science Research Council says that autonomous organizations are best able to judge the "intrinsic merit" of proposals for expenditure in education and research. It draws attention to the way in which its position outside the government proper enables it to call on the voluntary services of people "with the experience and qualifications to advise how public funds can best be used", pats itself on the back for its policy of selectivity and concentration and promises that it will be increasingly vigilant in trying to match the directions in which research and education are supported with what are called national needs.

The council says that the diminished pace of growth of expenditure will begin to restrict the council's activity in the coming financial year. The report says that "beyond March 1972, it is certain that the funds available will be such that it will not be possible for the council to carry out in full the programme which could be justified on educational scientific grounds". The council is worried that the university population will be growing more quickly

than its own resources and it says that there will be an even greater need for the determination of priorities in different fields of the council's work. The council is particularly anxious about long-term capital projects now urgently needed in Britain.

On strategy for research, the report says that it will continue to give priority to engineering and the applied sciences as well as to areas of research which have been picked out for their promise of intellectual and practical benefit. The council says that it will do what it can for astronomy and in particular help to build an observatory in the northern hemisphere, but that the cost of participating in the project to build a 300 GeV European accelerator will have to be accommodated, as every high energy physicist has known for more than a year, by reducing the cost of the domestic programme. On relationships with the universities, the council says it wishes no change in the present division of responsibility for government support of university research between itself and the University Grants Committee, but that in future the practice will be abandoned of transferring funds from the Science Research Council to the University Grants Committee for the support of research projects begun under the council's auspices.

There is also to be a relaxation of the rules which have so far restricted the numbers of people employed as research assistants with the help of research grants, although the council says that the duration of these research posts will be limited so as to persuade both the universities and the people concerned that more permanent arrangements would be in their best interests. In spite of the council's previous attempts to divert research students from PhD courses to other courses of advanced training, possibly as pre-liminaries, the number of new research studentships increased by seven per cent to 2,158 in 1970 and the number of awards for advanced courses by two per cent to 1,568.

The report says that for the first time the council had to turn away qualified applicants in engineering departments. In 1970, 32 per cent of its research studentships were awarded to people in applied science departments, but since 1969 the pace of growth has been faster in the pure science departments (10 per cent) than in the applied science departments (5 per cent). There has, however, been a substantial increase (from 180 to 200) of the cooperative awards in pure

science by means of which people working in industry are enabled to carry out research at a university. The council is also pushing hard for different kinds of PhD courses, preferably with a broader base. The Engineering Board of the Science Research Council seems to have been especially active in the past year in assessing what it calls the "total technology" needs of young engineers.

Expenditure by the SRC in 1970-71 amounted to £51 million, an increase of £4 million on the previous year. The Rutherford Laboratory remains the largest of the council's domestic pensioners (£8 million in 1970-71), the Daresbury Laboratory with its electron accelerator comes next (£4.5 million) and the Atlas Laboratory and the Radio and Space Research Station cost £1.16 and £1.63 million respectively. Grants to universities cost a total of £9.65 million, with engineering the most generously treated (£3.89 million), nuclear physics still keeping its head above water with £1.56 million and the Astronomy, Space and Radio Board generously increasing its expenditure by more than 20 per cent to £1.12 million.

The report welcomes the international collaboration which allows British scientists to use facilities abroad and enabled 220 foreign scientists to spend up to a year in Britain last year.

The report is also enthusiastic about collaborative programmes, drawing particular attention to the UK interest in the Institut des Hautes Etudes Scientifiques, the proposed use of the Franco-German High Flux Beam Reactor at Grenoble and the Radio and Space Research Station link with the Italian satellite project SIRIO, and the SRC is particularly delighted that the government—at the SRC's consistent recommendation—has agreed to UK participation in the CERN 300 GeV project.

Disappointment is expressed that the discussions on the future of the European space programme have so far been unproductive, no agreement being reached on either a future European programme or on European involvement in NASA's post-Apollo programme. But the SRC is encouraged by Britain's individual involvement with NASA, citing the presence of American experiments in the payloads of the UK-4 and UK-5 satellites, the provision of experiments for the American programme by British scientists and the continuing analysis of lunar samples in Britain. The SRC also welcomes the possible future involvement in the ultra-violet astronomy satellite SAS-D.

NEW WORLD

DDT Nailed Again

by our Washington Correspondent

A SCIENTIFIC advisory committee appointed by William D. Ruckelshaus, Administrator of the Environmental Protection Agency, has added its voice to the chorus of opposition to the use of DDT in the United States. Five top level scientific committees in almost as many years have recommended that the pesticide be phased out of domestic use as soon as possible, but in spite of the armoury now available to pesticide critics, the battle of words over the safety of DDT is far from finished.

So far this year, DDT has been the subject of two decisions of the US Court of Appeals, its use in the United States has been cancelled by the Environmental Protection Agency (EPA), that decision has been appealed against by chemical manufacturers, a lengthy public hearing has been started and a scientific advisory committee has issued a report. These skirmishes are, however, the result of a skilful rearguard action being fought by pesticide manufacturers to delay a ban on DDT, and there seems little likelihood of anything other than final victory for the anti-pesticide forces.

The first indication that the tide had turned against DDT came in November 1969 when the Department of Agriculture cancelled four major uses of the pesticide in the United States—on tobacco, shade trees, over water and in the home. Spurred on by the taste of blood, environmental groups led by the Environmental Defense Fund initiated a sequence of court actions which culminated in a decision of the Court of Appeals in January this year directing the EPA to cancel all remaining uses of the pesticide, and to consider suspending its registration (immediate suspension would prevent DDT from being marketed while the cancellation process takes place).

The EPA obediently cancelled all uses of DDT in the United States but concluded that suspension is not warranted, thereby setting in train a public hearing, another court action and a study by an advisory committee. The public hearing and the setting up of the advisory committee were requested by manufacturers exercising their rights under the Federal Insecticide, Fungi-

cide and Rodenticide Act to appeal against a cancellation order, and the court action was brought by the Environmental Defense Fund in protest against the EPA's decision not to suspend registration of DDT. The upshot of the bureaucratic manoeuvring so far is that the advisory committee has recommended rapid phasing out of all uses of DDT, but that the pesticide should continue to be made available to the public health service for "control of disease-bearing insect vectors until satisfactory alternatives are developed", the appeals court has instructed Ruckelshaus to reconsider his decision not to suspend registration of DDT and to make a final decision by November 1, and the public hearing is likely to drag on until next year.

Ruckelshaus has ninety days in which to respond to the advisory committee report, and with two court decisions to back him up, he is unlikely to revoke the cancellation order. But if he is looking to the report for inspiration to help decide the vexed problem of suspension he will be disappointed, for on the imminence of the hazards associated with use of DDT, the advisory committee is skilfully ambiguous. It suggests that present use of DDT "does not present an imminent hazard to human health, in terms of bodily functions and safety", but continued use of DDT is "an imminent hazard to human welfare in terms of

maintaining healthy desirable flora and fauna in man's environment". Even if all present uses of DDT were outlawed, however, the committee points out that because of the present levels of the pesticide and its persistence in the environment, a rapid decrease in the use of DDT will probably achieve the same result as suspension.

The committee bases those observations on the finding that in spite of the heat generated by the battle of words over DDT, there is little evidence that it is acutely toxic to man, no evidence that it is teratogenic and, on the basis of present intake of DDT and its metabolites, "the probability of tumorigenesis and carcinogenesis is low". The case against DDT therefore rests essentially on its effect on non-target species and on the ecosystem itself, and here the committee is able to cite a sheaf of undesirable consequences of widespread use.

By far the most damaging behaviour of DDT is that it can remain in the environment for several years in its original form or as the metabolites DDD and DDE. The result is that in spite of a rapid decline in the use of DDT in the United States during the 1960s (from about 70 million tons a year in 1962 to about 30 million in 1969), there has been no parallel decrease in the contamination of the environment. Levels of DDT in the soils of south-western, southern and

Table 1 Predicted Concentration of DDT and DDE in Human Adipose Tissue with Various Assumptions about DDT Usage

Year	Continued reduction of DDT usage at present rate	Zero future usage of DDT	DDT usage maintained at 5×10^8 lbs./year	DDT usage maintained at 1966 levels
1970	5.14	5.14	5.14	5.30
1974	4.18	4.13	4.20	5.08
1978	3.41	3.35	3.53	5.32
1982	2.78	2.73	3.02	5.60
1986	2.27	2.23	2.60	5.84
1990	1.86	1.82	2.25	6.03
1994	1.52	1.49	1.98	6.20
1998	1.24	1.21	1.75	6.32
2002	1.01	0.99	1.56	6.43
2006	0.82	0.81	1.41	6.52
2010	0.67	0.66	1.29	6.59
2014	0.55	0.54	1.18	6.65
2018	0.45	0.44	1.10	6.70
2022	0.37	0.36	1.03	6.73

eastern seaboard states still stand at between 0.10 and 0.56 parts per million, although in orchard soils they have been found to reach 52 ppm, and DDT is still found in rivers and lakes at concentrations approaching the limits of its solubility.

What seems to be happening is that run-off from agricultural soils has pumped DDT into the rivers to the extent that it has settled in bottom sediments. This creates a pool of DDT which is stirred up and released into the atmosphere by storms, and the rivers are continually being recharged by run-off from the soil. The upshot is that there is no prospect of marked decrease in water contamination until levels in soils and sediments are decreased—a process that will take years even if no more DDT is pumped into the ecosystem of the United States.

The chief cause of concern over the continuing contamination of the marine environment by DDT is that its high solubility in body fats causes it to accumulate in the tissues of animals in the higher levels of the food chain, sometimes with devastating consequences for animal populations. Evidence that the decline in populations of the peregrine falcon and bald eagle is linked to the build-up of DDT or its metabolites in their tissues is already well documented, and a committee of the National Academy of Sciences concluded earlier this year that marine fish are almost universally contaminated by chlorinated hydrocarbon residues. What happens is that phytoplankton accumulate DDT from water, and the pesticide then finds its way into the tissues of fish, and so on up the food chain. An example of how the levels of DDT become magnified in living tissue is the fact that half of the fish caught in Lake Michigan in 1966, for example, had at least five parts per million of DDT in their tissues, compared with 0.0012 parts per million in the water.

Although that sequence of contamination has been drummed into the public through the protestations of environmentalists for the best part of a decade, the committee nevertheless complains of a lack of hard evidence on which to base predictions of the body burden of DDT in future years. Present evidence indicates that a man consumes about 0.01 mg of DDT or its metabolites a day, and has about 6 ppm of DDT and DDE in his adipose tissue, but while DDT intake has been more than halved since 1966, tissue concentrations have declined by less than 25 per cent.

The committee believes that levels of DDT in food are now in equilibrium with levels in the environment, and since the environmental burden is unlikely to decline very rapidly, even if no more DDT is used in the United

States, food levels will probably remain fairly constant over the next few years. Moreover, since average concentrations of DDT in human tissue have not yet reached equilibrium with the concentrations in food, they will continue to remain high for some time to come. These predictions are, however, necessarily hand waving extrapolations from incomplete data, and the committee decided to explore the possibility of devising a simple systems model to predict the level of DDT metabolites in man up to the end of the decade.

A study carried out by R. V. O'Neill and O. W. Burke of the Oak Ridge National Laboratory concluded that even if no more DDT is used in the United States, the adipose tissue of Americans would still contain 0.36 ppm by the year 2022. Moreover, tissue levels of DDT will decline at almost identical rates if DDT usage continues to decline at its present rate, or if the pesticide is removed completely from the US market. A small use of DDT will, however, have a marked effect on tissue concentrations at the end of the century (see Table 1).

Is the alarm engendered by the build up of DDT in human tissue matched by evidence of its chronic toxicity? The advisory committee believes not, and it bases its *sang froid* on the fact that there is more information on the toxicity of DDT than on any other pesticide, and yet there have been no well documented reports of fatal uncomplicated DDT poisoning and there is little evidence of carcinogenicity at the levels encountered by man—although the committee is quick to point out that firm conclusions cannot be drawn.

The committee cites studies carried out in 1956 which showed that rats fed DDT in doses of 15 ppm produced hypertrophy, inclusion bodies and cytoplasmic granulation in the liver, but that these changes were reversible after withdrawal of the diet. A later study by the Bionetics Research Institute, and studies carried out under the auspices of the International Agency for Research on Cancer, both showed a statistically significant increase in the incidence of hepatomas, and a study carried out in 1967 produced evidence of hepatic cell tumour in trout fed relatively low doses of DDT. These results have led the advisory committee to conclude that "the evidence to date clearly shows that DDT induces hepatomas and suggests it may be carcinogenic", but studies of workers in DDT factories, where average daily intake of the pesticide probably reaches about 18 mg, have produced "no clinical or laboratory effects attributable to exposure to DDT". Nevertheless, the committee suggests that long-term studies of occupational exposure to DDT are urgently needed, and that in spite of the evidence

that there is little cause for alarm from health hazards, the possibility of carcinogenicity cannot be dismissed.

As for toxicity to animals and birds, however, the committee points out that data are incomplete and further research is needed to support definite conclusions. Nevertheless, there is sufficient circumstantial evidence to link build up of DDT levels in some species with their decline in numbers, and "there is sufficient toxicological information on DDT in aquatic species to indicate that reduction and prevention of contamination of water sources is a problem of major concern".

Materials Regrouped

by our Washington Correspondent

REPERCUSSIONS of the so-called Mansfield Amendment, directing the Department of Defense to keep its nose out of research not directly related to weapons development, are still being felt in the National Science Foundation. Apart from being given an extra \$40 million in its budget to pick up projects dropped by the Defense Department, the NSF has inherited responsibility for so much materials science that it has decided to re-group its support programmes. A new division of materials science has been set up to look after the twelve interdisciplinary research laboratories acquired from the Advanced Research Projects Agency—a defence department agency designed to get advanced projects moving (it was at one time responsible for the Arecibo telescope)—and the National Magnets Laboratory that was once the responsibility of the Air Force. The new division will also take charge of the materials science activities that were funded by the other divisions of the NSF. The foundation last year distributed grants worth about \$30 million for materials science, and its stake in the field is expected to double this year.

The director of the new division is Dr Harold W. Paxton, recruited to the NSF from the metallurgy department of Carnegie-Mellon University, and the deputy director is Dr Howard Etzel, formerly director of the NSF's Solid State and Low Temperature Physics Program. The chief problem facing the division at present is that although it is already formally constituted, it has run into staffing troubles because of restrictions imposed on government agencies by the wages and prices freeze.

These conclusions have led the committee to recommend that the use of DDT be phased out, but that it remains available for the control of disease bearing insects at least until satisfactory alternatives have been developed — a conclusion that has already been interpreted by the court of appeals as calling for suspension of the pesticide, and by some manufacturers as an acceptance of their thesis that DDT is the safest pesticide to use in most applications. Although the days for DDT in the United States are clearly numbered, there is no sign as yet that it will be allowed to pass peacefully.

MANPOWER

A Modest Plan

by our Washington Correspondent

THE Department of Labor has put up \$3 million to help find work for highly qualified scientists and engineers who are at present swelling the ranks of the unemployed. The money will be used to establish internships in federally funded laboratories for some 420 young unemployed scientists and engineers who hold advanced degrees. A brainchild of the Office of Science and Technology and the Department of Labor, the scheme is being administered by the National Science Foundation, and it is expected to swing into action in the near future.

The idea was sketched out in a memorandum addressed to all directors of federally funded laboratories with more than 100 professional staff on their payroll, sent out last week by Raymond L. Bisplinghoff, acting director of the NSF. It explained that the foundation will provide \$7,000 to pay the salary of each intern on a one year non-renewable basis, provided that the laboratory at least matches that sum to supplement the salary and to meet overhead costs. Laboratory directors have been asked to furnish the NSF with an estimate of the number of internships which can fruitfully be used to the mutual advantage of the laboratory and the intern, and the foundation will parcel out 420 internships on a first-come first-served basis. Interns will then be recruited by the laboratory with preference being given to veterans and those from areas of particularly high unemployment, which in general means areas in which defence and aerospace industries are concentrated.

Edward E. David, the President's science adviser, said last week that he hoped that the interns would be employed in positions "where they can best benefit the nation and themselves" — projects concerned with pollution, rubbish disposal, nuclear energy and the like. But the NSF memorandum made

no mention of any constraints on the type of project except that care should be taken "in assigning interns to tasks which will improve their potential for later job placement".

The scheme is an offshoot of a \$42 million programme launched by the Department of Labor in April to help find work for scientists, engineers and technicians thrown out of work by cutbacks in the defence and aerospace industries. The thrust of the programme is to provide retraining for about 10,000 people who have a strong possibility of being employed by specific employers, relocation grants for scientists and engineers who find employment out of their home area, and to set up a national registry to help match jobless engineers with job openings. These facilities are limited to applicants from fourteen areas particularly

hard hit by unemployment, unlike the internships scheme, which is limited neither by the area in which the applicant is living, nor by his previous employment. One reason for the difference between the schemes may be that the NSF has been able to dictate the terms of the internship project.

The new initiative by the Administration in trying to find employment for some of the jobless scientists and engineers is probably a measure of the political embarrassment in having expensively produced graduates in the dole queues during election year. But the internship scheme is hardly likely to be a decisive influence on the problem, for according to a survey conducted by the NSF, some 4,000 scientists and engineers holding a master's degree or a doctorate were out of work in the spring of this year.

Short Notes

FAS on the Attack

THE Federation of American Scientists has launched a stinging attack on the F-14 carrier-based aircraft programme. A statement issued by the FAS describes the programme as "a purposeless, enormously expensive, gold-plated procurement scandal dependent on a missile that may not work; its only hope is to become a *fait accompli*". The idea of the programme is to provide about 300 aircraft to serve on carriers in "high threat areas", in an effort to protect them from enemy action, but the FAS—which has in the past been a consistent critic of federal arms policy—suggests that carrier-launched attacks are unlikely to be effective in Soviet wars, and that land-based aircraft are unquestionably superior in terms of both capability and cost.

The military appropriations request for 1972 is for \$1,034 million for 48 aircraft—a figure which the FAS points out would result in a cost for 300 aircraft of \$6,000 million, allowing for research and development on the first batch. "It is a self-evident distortion of priorities to give carriers marginally improved abilities to do a largely unnecessary and hopeless job," the FAS tartly states. The federation also calls the procurement of the F-14 a scandal because the House of Representatives has had no chance to debate the programme. It charges that the chairman of the Armed Services Committee held back the procurement request pending a review by Mr Packard, Deputy Secretary of Defense, of the number of aircraft to be bought, but long before the conference committee report is debated, the Administration will sign a contract for production of 48 aircraft.

Davis lashes OMB

JOHN W. DAVIS, chairman of the House Subcommittee on Research and Development, has lashed the Office of Management and Budget's decision to impound \$30 million of the National Science Foundation's funds for support of science education. The OMB's action, which has already led to the abrupt resignation of Lloyd Humphries, an assistant director of the NSF, was cited by Davis in a letter to President Nixon as likely to endanger the "long run health of American science". Davis asked Mr Nixon to take immediate steps to remedy the OMB's action. The subcommittee on Research and Development specifically increased the NSF's budget authorization for science education, and it is naturally chary of having its work negated by a decision of the Office of Management and Budget, taken behind closed doors.

Aggressive Bees

A COMMITTEE of the National Research Council has been set up to plan methods to cope with the possible intrusion into Central and North America of a particularly aggressive strain of honey bee. The bee was accidentally introduced into South America 15 years ago during experiments designed to breed into the ordinary domestic bee the extraordinary work capacity of the African honey bee *Apis mellifera adansonii*. Unfortunately, however, the African bee, which has a painful sting and will sometimes attack when unprovoked, escaped and spread rapidly over South America. Beekeepers in North America fear that if the African bee spreads to North America, its viciousness might lead to demands for eradication of all bees near urban communities.

NEWS AND VIEWS

Cancer and Immunity

It would be hard to find a cancer researcher who would these days deny that the state of an animal's immune defence system crucially affects its response to carcinogens and transplanted cancer cells or that the growth of cancer cells into a tumour is correlated with at least a partial failure of the immune system. Indeed it has been seriously argued that the immune system first evolved as a defence mechanism against autochthonous tumour cells and only secondarily became adapted as a defence against infecting microorganisms and other foreign antigenic materials arriving from outside the individual. But if one of the chief functions of the immune system is to provide a surveillance mechanism which can recognize and destroy cancer cells because they carry tumour antigens on their surfaces which are not present on normal cells, how do tumours ever develop? In other words, by what mechanism is the immune system undermined to such an extent that tumours can grow and metastasize?

In an attempt to shed some new light on this crucially important question, Ambrose, Anderson and Coggin at the Oak Ridge National Laboratory have analysed the role of circulating antibodies during the development of tumours in hamsters injected with Simian Virus 40. And as they report on page 321 of this issue of *Nature*, they believe that cytostatic antibodies play an important part in tumorigenesis, during the early stages of which they seem to prevent the rapid proliferation of the initially arising tumour cells. Once the tumour, in the face of these antibodies, reaches some critical mass, however, tumour antigen becomes locally in excess, swamps the available antibody and cell mediated immunity and uncontrolled growth ensues.

In spite of its limitations, Ambrose and her colleagues chose to use as their model system Simian Virus 40 as a carcinogen for baby hamsters for three chief reasons; first, the virus reproducibly induces tumours; second, all SV40 induced tumours bear the same transplantation antigens; and third, and most importantly, it is possible to block the induction of tumours by immunization with either SV40 virus or SV40 tumour cells killed by irradiation.

The first problem encountered by Ambrose *et al.* was to devise ways of distinguishing the effects on tumorigenesis of humoral or antibody immunity responses from those of cellular immunity mediated by sensitized lymphocytes. That cellular immunity plays an important part in the defence against cancer is, of course, indisputable—non-immune animals, for example, can be protected against tumour cells by injections of lymphocytes from immune animals—but the role of antibody immunity is much more obscure and is something Ambrose *et al.* were intent on elucidating. To test whether antibodies as well as lymphocytes in animals immune to SV40 tumours can kill or at least prevent the proliferation of SV40 tumour cells, they placed such cells in plastic chambers permeable to antibodies in the peritoneal cavities of immune and non-immune hamsters. Circulating antibodies in the immune animals, but not those in the controls, retarded or prevented the proliferation of the cells.

The Oak Ridge group then exploited this technique to follow the course of appearance of these cytostatic, if not cytotoxic, antibodies during the latent period between the injection of SV40 virus into newborn hamsters and the appearance of tumours months later. During the first ten weeks of life the amount of circulating cytostatic antibody increases and in those animals which do not develop tumours, even after 500 days, antibody persists, but in those animals fated to develop tumours the amount of this antibody declines precipitously after about ten weeks. These findings suggest that during the first ten weeks after injection with SV40, transformed tumour cells are present but latent and indeed such cells can be detected by histopathology. Furthermore, by incubating SV40 tumour cells with sera from normal hamsters, immune hamsters and tumour bearing animals, Ambrose and her colleagues proved that sera of immune animals but not tumour bearing animals prevent the multiplication of tumour cells *in vitro*.

There can be no doubt that circulating antibody from immune animals is cytostatic, but sera from animals with tumours lack this material. A further set of experiments established three important facts about this antibody: first, it seems to be invariably produced before tumours appear; second, it retards tumour growth; and third, it is always present in immune animals. And if the transient appearance of the antibody precedes the appearance of tumours, what happens when tumours are removed by surgery; does the antibody reappear? As might be anticipated, it reappears in at least some animals.

What is the simplest interpretation of all these data? Ambrose and her colleagues, who firmly believe as a result of their own experiments as well as those of others, that the so-called tumour specific transplantation antigens are probably normal early embryonic antigens caused to be re-expressed by transformation, draw an analogy between the tolerance of a foetus and the survival of a tumour. They believe that immediately after exposure to a carcinogen, a small tumour mass is established and may for a few hours grow without restriction. But as cytostatic antibodies are produced the rate of proliferation of surviving tumour cells is greatly reduced and during the latent period the tumour mass increases only gradually. When, however, a critical mass is reached the cytostatic antibodies are locally mopped up by an excess of tumour antigen. The tumour cells can then grow rapidly producing more antigen and as this excess builds up circulating antibody becomes undetectable and amounts of antigen always outweigh amounts of antibody, unless of course the tumours are removed by surgery. And once antigen is in excess, antigen-antibody complexes may block cellular immunity and even cause immune enhancement.

This scheme clearly contains many elements of speculation but it is nonetheless attractive and suggests that assays for antitumour antibodies, be they cytostatic or cytotoxic, might provide an early warning system for human cancers still in their latent period and an indication of when treatment of established tumours is being successful.

NUCLEIC ACIDS

Genes Made Manifest

from our Molecular Biology Correspondent

ONE of the prettiest techniques to bubble up from the cauldron of molecular biology in recent years is Inman's denaturation mapping of DNA. The DNA is brought to a precisely defined temperature, or pH, at some point within its narrow melting range. Formaldehyde is introduced to prop open the melted parts of the chain, and a population of molecules so treated is examined in the electron microscope. The denatured regions are then indexed along the chain for a sufficient number of molecules on the grid, and correspond to tracts of sequence relatively lower in (G+C) than the rest. An interesting new application for this approach has now been found by Wensink and Brown (*J. Mol. Biol.*, **60**, 235; 1971), namely the identification of a gene, using its characteristic denaturation map as a fingerprint.

It is known that the DNA corresponding in eukaryotic cells to the ribosomal RNA is present in the form of many clustered copies, apparently interspersed by spacer segments, much richer in (G+C). Moreover this ribosomal DNA is separately amplified, and occurs as an extra-chromosomal component, differing apparently only in that none of its cytosine residues is methylated. Such a DNA, when subjected to the denaturation mapping technique, displays in the electron micrographs an immediately apparent periodicity, repeating up to fifteen times in one molecule. To ensure, however, that this effect is no trick of the eye or mind, Wensink and Brown have devised a means of testing for the presence of a periodicity and determining its precision and spacing. The measured distribution is matched against a Fourier function, the wavelength of which is varied until a correlation appears and is maximized. A sharp peak comes up at a periodicity of $5.4 \pm 0.4\mu$, whereas a denaturation map of T2 phage DNA shows only a flat background. From a calibration of the length measurement against T7 DNA, as a molecular weight standard, the repeating unit turns out to correspond to a molecular weight of some 8.7×10^6 which is the same as the molecular weight determined by Dawid and his colleagues of 9×10^6 .

The repeating unit consists of a rather readily denaturable region, and another, somewhat shorter and much more resistant to melting, which may therefore be surmised to be richer in (G+C). This is exactly what would be expected from the size and composition assigned to the spacer region between successive copies of the ribosomal RNA gene. A comparison with Inman's results on λ phage DNA, which can be divided into two

halves differing by 20 per cent in their (G+C) content, indicates that there is a similar difference between the ribosomal RNA tract and the spacer.

This is a very satisfactory result, but there is still more information in the denaturation maps, for the map of the low-melting segment contains a highly reproducible fine structure, which persists over a wide range of melting. Dividing the repeating unit into 100 elements, a histogram of the fraction of chains in which each element is melted shows a relatively broad region of melting, followed by a trough, corresponding to a much more resistant sequence, then another, narrower peak, followed in turn by a narrow and extremely resistant minimum, and then a mirror image of the first pair of separated peaks. The pattern is thus symmetrical about a short, highly (G+C)-rich segment on which the patterns are most readily indexed. This whole pattern corresponds to the 5×10^6 molecular weight of the 40S ribosomal RNA precursor, which contains the 28S and 18S chains of the large and the small subunit. In conversion of the precursor to these products a small portion of the chain is lost. This portion is believed to be rather rich in (G+C), and may be presumptively identified with one of the two shallower troughs in the denaturation map. This latter must therefore separate the 28S and 18S parts of the chain. The short very stable region can also be accounted for, because partial enzymatic degradation of

28S ribosomal RNA from other animal cells has led to the isolation of a sizable fragment (200,000 molecular weight) containing no less than 80 per cent (G+C). Wensink and Brown have compared finally the denaturation maps of the chromosomal and amplified ribosomal RNA, and found them to be identical.

Lord Kelvin's criterion that only that which can be measured and expressed in figures is science, can also be applied to another interesting essay in electron microscopy. Freifelder (*ibid.*, 401) has given experimental proof to the proposition that intercalative binding in DNA causes a change in the pitch of the double helix with a consequent increase in length. Using monodisperse phage DNA he has measured the length distribution of the molecules as a function of the concentration of added ethidium bromide. The standard deviation of measured lengths in each binding state is only 3 per cent, and the increase in length with ligand concentration reaches a sharp saturation level. At this point the chain has stretched by some 27 per cent. Moreover, the molecules look quite different: instead of a rather crinkly thread, broadly curved filaments are seen. Circular DNA-dye complexes have the appearance of smooth ellipses. It seems then that the hydrodynamic stiffness of the molecule must change on intercalation, so that direct inferences about the length from viscosity or sedimentation measurements are not in fact justified.

Locating a Quasar

OUTSIDE the circle of observational astronomy it sometimes comes as a surprise to learn that the exact positions of celestial objects are not always known, and in particular that there is considerable difficulty in relating positions measured by optical and radio techniques. A little thought, however, makes it seem more remarkable that identifications at optical and radio frequencies can be compared at all in some cases, at least to the accuracy required before theories can be built upon the observations. Even optical positions can only be measured relative to certain reference stars, and at radio frequencies sources can only be pinned down by a technique such as occultation, or, most commonly, by finding an optical counterpart to the source. The embarrassing aspect of the second technique is that it is less than adequate when the object of the exercise is to determine whether an optical source and a radio object are one and the same.

A classic example of the success of the occultation technique was provided by the identification of the source 3C 273, the first known quasar, by timing the

eclipse of the radio source by the Moon. But it has proved impossible, until now, to state unequivocally that the small component 3C 273B really is coincident with the associated QSO, because of a difference of 0.7 arcsec between the best optical and radio positions published. Dr C. Hazard and his colleagues at the University of Cambridge and the Royal Greenwich Observatory have rectified this situation, and publish in next week's *Nature Physical Science* positions which confirm the association of the radio and optical objects to within 0.3 arcsec. Unlike the earlier recorded difference (0.7 arcsec) this figure is well within the limits of experimental error. Indeed, the best of the lunar occultation observations gives a radio source position exactly at the mean of the optical positions. Because of the differing reference systems, however, such good agreement is not to be taken at face value. There is no reason to prefer either the radio position or the optical position, so that the best position now available for 3C 273B should be taken as the average of the two positions determined by this work.

COSMIC RAYS

Hobart Conference

from a Correspondent

THE twelfth international conference on cosmic rays was held for the first time in the southern hemisphere at the University of Tasmania, Hobart, from August 16 to 25, 1971. In spite of the large distances involved for most participants delegates from twenty-two countries, not including Australia, attended.

In the high energy sessions the delegates heard of further experiments that had been carried out in the search for quarks and other particles. The contributions did not advance the position, however, and no conclusive new evidence for the existence of these particles was presented. The Sydney quark experiment has not been run significantly since the Budapest conference and no new events were reported. The experiment is currently being refurbished by the inclusion of five high pressure cloud chambers.

At the Calgary cosmic ray conference in 1967, the University of Utah group reported that their underground measurements on the variation of cosmic-ray muon intensity with zenith angle, θ , in the TeV range, showed no "sec θ " enhancement as predicted by theories involving pion parentage. It was suggested that this could be explained in terms of muon parents of very short lifetime, subsequently referred to as X-particles by the Utah group. The observations of the Utah group have continued and have been refined but the onset of the effect seems to have receded to somewhat higher energies and reaches a maximum, only to disappear again at even higher energies. Other experimenters, notably the group at the Tata Institute working in the Kolar goldfields, continue to find no evidence for a departure from the sec θ enhancement. It would seem that perhaps a third independent experiment at a depth of about 1,500 hg cm⁻² should be carried out to try to resolve these apparently contradictory results.

Details of several large solid iron muon spectrographs being built or recently completed in order to study this effect directly were given at the conference. But the problems associated with the measurement of muon energies of about 3 to 5 TeV, coupled with the low flux of particles at these energies, mean that some time must elapse before these instruments will be able to contribute to an elucidation of this problem.

Although extensive air showers have been studied for several years, the origin and nature of the primary particles are not yet well understood. At the Jaipur cosmic ray conference in 1963 the results of the MIT (Volcano Ranch) group suggested that the slope of the integral primary energy spectrum

changed from 1.6 (below 10¹⁶ eV) to 2.2 (between 10¹⁶ and 10¹⁸ eV) and back to 1.6 at energies greater than 10¹⁸ eV. These "ankles" were tentatively interpreted as reflecting a change in the mass composition of the primary cosmic-ray particles. The part of the spectrum beyond the second ankle was thought to be attributable to primary protons of extragalactic origin.

Perhaps the most significant change at the Tasmanian meeting was the report from the University of Leeds group working at Haverah Park in Yorkshire. These results showed no evidence for the existence of the second ankle. Furthermore, it was shown that by applying the same method of analysis to the MIT Volcano Ranch data, the evidence for the second ankle disappeared.

Preliminary data presented at the conference from the Sydney University Giant Air Shower Recorder (SUGAR) in the form of muon size spectra also showed no evidence for the second ankle at 10¹⁸ eV. Should these results be substantiated they will constitute an important observation which could lead to queries about the existence of extragalactic radiation. Measurements of the arrival directions of the most energetic showers yield no evidence of any anisotropy even when the galactic centre

is overhead.

Several speakers described attempts to link the observed shower properties at sea level with the mass of the primary particles. Some progress in this field was reported by the Imperial College and University of Nottingham groups. It would seem that studies of the electron and muon component coupled with the observations of the radio emissions from showers might well be able to give more definitive answers.

Direct studies of the mass composition of the low energy part of the primary spectrum have continued. By using new techniques including superconducting magnets, knowledge of the light/medium element ratio (L/M) has been extended up to 10,000 MeV per nucleon. It has been found that the value is approximately 0.2, and that the composition at the higher energies is equivalent to that at the lower energies.

Studies of elements with $Z > 26$ have shown that the abundance of uranium is greater than expected. The disagreement between the values of Z obtained from nuclear emulsion and plastic detectors has been resolved since the Budapest conference with the exception of one event. Studies of the light and heavy nuclei suggest that the age of the cosmic radiation is between 10⁶ and 10⁷ yr.

Arabinose Operon *in vitro*

IN *Nature New Biology* next week two groups, Zubay, Gielow and Englesberg and Greenblatt and Schlieff, report the successful reconstruction *in vitro* of the regulatory system which controls the expression of the arabinose operon of *Escherichia coli*. Their experiments are notable, for this is the first time that a positive regulator protein which causes the expression of a specific operon has been made to function *in vitro*. Furthermore, these *in vitro* experiments confirm the picture of the regulation of the arabinose operon which has emerged from studies of intact *E. coli* and they provide a method for assaying for the elusive arabinose regulator protein.

Zubay and his colleagues used the cell free system developed by his group to support the regulated expression of the *lac* operon to define the elements required for the synthesis *in vitro* of L-ribulokinase when the system is programmed with phage θ 80dara DNA which includes the arabinose operon. They find that in addition to cyclic AMP, the activator protein which binds cyclic AMP and ppGpp, cell extracts containing the protein specified by the *araC* gene are required together with arabinose. Genetic analyses had indicated that the *araC* gene protein can act both as a repressor and an activator of the arabinose operon and these

in vitro experiments confirm the latter function of this protein.

Greenblatt and Schlieff have, like Zubay and his colleagues, exploited mutants of the *araC* gene to prove that the *araC* protein is an activator *in vitro* of the arabinose operon. Moreover, they have shown that under certain conditions the *araC* protein can act *in vitro* as a repressor of the arabinose operon. These findings fully confirm the idea that the *araC* protein can exist in two functional states, repressor and activator, in equilibrium and that an inducer such as arabinose favours the activator form. Greenblatt and Schlieff have also reached the interesting conclusion that the protein specified by constitutive mutants of the *araC* gene can activate the arabinose operon *in vivo* in the absence of arabinose not because it is "frozen" in the activator state but because it can respond not only to arabinose but also to some as yet unidentified metabolite always present in growing cells.

Attempts to purify the *araC* protein by virtue of its ability to bind to arabinose or arabinose operon DNA have all failed, but now that the cell free system for ribulokinase synthesis provides an assay for this protein, its purification can be anticipated and that will open the way to a detailed analysis of a regulatory molecule.

MINERAL RESOURCES

New Prospects

from a Correspondent

TYNAGH, Gortdrum and Silvermines are household words in metal mining today. All are mines in the Republic of Ireland which have been brought into production within the past decade and are likely to be followed by others in the near future. Great Britain can point to a major new tin development, the Wheal Jane mine in Cornwall, and hopes are high for potash, copper and nickel elsewhere in the country. Both Ireland and Britain are being explored for metaliferous minerals on a scale and in ways never seen before. Their geologists and mining engineers came together in Edinburgh on September 10 to describe the new findings and the methods of exploration at a symposium arranged by the Institution of Mining and Metallurgy as one of a group of earth science meetings held to celebrate the centenary of the University of Edinburgh Geology Department.

With so much ore already proved, the geologists working in Ireland inevitably had much to say. Dr R. W. Schultz (Northgate Exploration Ltd, Dublin) gave high marks to geochemical soil and drainage surveys in his analysis of exploration practice. Although reserving judgment on the origin of mineralization in the Lower Carboniferous of central Ireland, Dr C. J. Morrissey (Irish Base Metals Ltd, Dublin), Professor G. R. Davis (Imperial College, London) and Mr G. M. Steed (Imperial College, London) made a lucid comparison of the characteristics of these valuable lead, zinc and copper deposits. In England, the Carboniferous is receiving most attention for fluorite, and Dr T. D. Ford (University of Leicester) and Dr P. R. Ineson's (University of Sheffield) comprehensive account of the Derbyshire orefield will simplify the task of future exploration in that area. The Lower Palaeozoic rocks of Wales, eastern Ireland, the Lake District and southern Scotland together form a major exploration target and the recognition by Mr C. J. V. Wheatley (Imperial College) of an axial belt of copper deposits associated with acid volcanic rocks and bordered by lead-zinc mineralization provides a highly stimulating basis for considering the controls of metallization within them.

The rapid re-development of the Wheal Jane mine into a major tin producer has surprised many people. There is clearly more mineral wealth in Cornwall than is contained in narrow tin lodes and the account of the structural environment of the deposit by Mr B. D. Rayment (Consolidated Gold Fields Ltd, London), Professor G. R. Davis and Mr J. D. Willson (Consolidated Gold Fields Ltd) will help to stimu-

late further exploration in south-west England. In the Scottish Highlands, reconnaissance investigations by the Institute of Geological Sciences have revealed new evidence of uranium, lead and molybdenum in the Old Red Sandstone sediments and Caledonian granites.

ENTOMOLOGY

Insect/Plant Relations

from a Correspondent

IN contrast to other recent contributions to the subject of insect/plant relations, the symposium held in London on September 16 and 17 by the Royal Entomological Society covered the field in breadth in a way which made it possible to appreciate the overall relevance and interrelations of disciplines ranging from biochemistry to ecology.

In introducing the symposium, Professor T. R. E. Southwood (Imperial College, London) considered relationships in terms of shelter, food and transport. He mentioned in particular the evolutionary hurdle which is seemingly presented by seed plants as food, because in spite of the great abundance of these plants they have been exploited by relatively few insect taxa. Within these taxa, however, the insect/plant systems have co-evolved from predation by the insect on the plant through parasitism to symbiosis.

Biochemical relationships were well exemplified by Dr D. Osborne's (ARC Unit of Developmental Botany, Cam-

bridge) contribution on mutual regulation of growth and development. Secondary substances in the plant may thus control development of the insect or even be needed to induce the female to emit its sex pheromone. Conversely, the insect may affect plant growth, not only by causing malformations but also by stimulating or delaying normal processes of leaf growth and senescence.

The Hon. Miriam Rothschild (Elsfield Manor, Oxford) discussed her work on warningly coloured insect species which sequester and store toxic substance from plants. This was a fascinating account of mechanisms which underlie forms of mimicry defined a century ago but, until now, inadequately examined biochemically. Miss Rothschild described how some tiger moths select only those host plants which retain certain alkaloids toxic to bird and mammal predators; the processes involved in such selectivity were examined by several other speakers. Dr L. M. Schoonhoven (Laboratorium voor Entomologie, Wageningen) described the chemoreceptors of a lepidopterous larva, which respond separately to a wide range of nutritional and secondary plant substances. He suggested that the basis for host plant specificity is more than mere recognition of particular plant substances and is dependent on the insect's ability to recognize the complex of many different substances associated with its own host plant and its nutritional requirements. Professor D. L. Wood (University of California, Berkeley) also described his research on

New Polynucleotide Structures?

BRAM has recently reported some unexpected findings on the dependence of DNA structure in solution on the base composition, and in next Wednesday's *Nature New Biology* he gives results of X-ray scattering studies on a set of two-stranded synthetic polynucleotides in solution. Leaving aside large differences in hydration or binding of counterions, differences in the low angle X-ray scattering envelopes from polynucleotides in solution should mean that they have differences in structure. The double-helical complex, poly dI.poly dC, shows a wide-angle scattering pattern which is different from that of A or B-form DNA. The axial radius of gyration, as derived from low-angle scattering, seems to be larger than that of B DNA and is interpreted to signify that the centre of gravity of the base pairs lies off the helix axis. The same is also found in poly dG.poly dC.

For the alternating double-helical polymer, poly d (I-C) the axial radius of gyration is similar to that of DNA and the alternating poly d (A-T). The

corresponding helix cross-section agrees with that found in fibres. Bram finds, however, that the wide angle scattering patterns of both this polymer and of poly G.poly C are different from any others found in polynucleotides or natural nucleic acid.

The double-stranded RNA analogue, poly rI.poly rC, gives scattering patterns which are compatible with the A-DNA-like structure (A'-form) of this polymer, found in fibres. Bram regards this agreement as support for the validity of experiment and the theoretical analysis. The generalization with the data so far available is that in double-stranded polynucleotides with purines and pyrimidines on opposite strands, the radius of gyration is larger than in alternating polymers, and the base pairs lie off the helix axis. Altogether Bram now identifies six different structures for DNA-type molecules. He suggests that the unique configuration of the poly G.poly C raises the possibility that parts of a natural DNA rich in these nucleotides could have a different structure from the rest of the chain.

a bark beetle which kills its host tree following the attraction of massive numbers of beetles to particular volatile substances in frass and resin produced by the activity of perhaps no more than one initial beetle colonist.

The behavioural relationships of an insect to its host plant were emphasized in Professor J. S. Kennedy's and Mr I. H. M. Fosbrooke's (Imperial College, London) discussion on the plant in the life of an aphid. They suggested that the migratory aphid's flight behaviour continues to be influenced importantly by light reflected from plants irrespective of the height at which the insect is flying.

In discussing the plant in the population dynamics of insects, Dr H. F. van Emden (University of Reading) and Professor M. J. Way (Imperial College, London) pointed out that even "chemosensory susceptible" host plants often cause surprisingly large direct mortalities to populations of insects established on them. They also emphasized the role of the plant in providing the framework within which subtle density dependent effects of intraspecific competition operate.

The role of the host plant in the population dynamics of phytophagous insects is still very inadequately understood—and vice versa—as was shown by Dr P. Harris (Canada Department of Agriculture, Belleville), who used examples of successful biological control of weeds to show how introduced phytophagous insects have severely limited the distribution and abundance of a few introduced plant weeds. In general, however, the insect's effect on population dynamics of indigenous plant populations still seems to be largely a matter for speculation except in circumstances where the plant has become dependent for pollination on certain insects.

PACIFIC SCIENCE

Island Conservation

from a Correspondent

THE occasion of the twelfth Pacific Science Congress, held at the Australian National University from August 18 to 27, was taken to celebrate the fiftieth anniversary of the Pacific Science Association which held its first meeting at Honolulu in 1920, under the presidency of its founder, Professor H. E. Gregory.

The congress was addressed by its president, Sir Macfarlane Burnet, after which the Gregory Fodal was presented to Dr F. Raymond Fosberg (Smithsonian Institution), the Shinkishi Hatai Medal to Dr Carl L. Hubbs (Scripps Institution for Oceanography) and honorary life memberships were conferred on Dr Sarwono Prawirohardjo (Indonesia) and Sir Maurice Yonge (Great Britain).

Much of the programme of the congress had relevance to the International Biological Programme with its emphasis on conservation. The resolutions submitted by the council at the final meeting reflected the general concern about the environment which, even in remote Pacific islands, is being increasingly degraded by the effects of pesticides, of mining, of the cutting down of primeval forests and, by no means least, the effect of tourism with its impact on the dignity of native peoples.

Such problems had been the particular concern of a regional symposium on the conservation of reefs and lagoons held before the congress, at Noumea, New Caledonia, under the auspices of the South Pacific Commission in collaboration with the International Union for the Conservation of Nature and Natural Resources (IUCN). A resolution coming from this symposium included reference to the urgent need for appropriate environmental and ecologi-

cal surveys being conducted before any "developmental" project. It also urged intergovernmental action on the imaginative "islands for science" proposal whereby a representative number of islands, largely uninhabited, could be set aside as permanent reserves unaffected by environmental changes induced by man.

It was also suggested that periodic surveys might be carried out on coral reef areas to record long-term fluctuations in the comparison of reef ecosystems. The importance of such knowledge is emphasized by the present widespread population explosion of the coral destroying starfish, *Acanthaster planci*. In this connexion, also, the Pacific Science Council established a new Committee on the Scientific Study of Coral Reefs for the better coordination of work on reefs which, for a variety of uncertain reasons, are now widely endangered.

New Normal Geomagnetic Event

DETERMINATION of the detailed geomagnetic polarity-time scale from continental igneous rocks of the past four million years or so has proved a comparatively simple task, partly because the polarities of young fresh rocks unaffected by appreciable continental drift are determined easily and unambiguously, partly because radiometric dating of such rocks is less likely to be affected by such factors as mineral alteration, and partly because within this period the absolute errors of the potassium-argon dating process are small enough to enable polarity intervals of as short as a few tens of thousands of years to be resolved. For older rocks these advantages are lost; and so it seems unlikely that the detailed pattern of magnetic reversals will ever be delineated for tens or hundreds of millions of years, lest it be on the basis of ocean magnetic anomalies. The essential problem is that even if continental rocks ideal for radiometric dating and magnetic measurement are found, the inherent dating errors will always be larger than all but the longest geomagnetic polarity intervals.

But as Creer *et al.* point out in next Monday's *Nature Physical Science*, there is one geological period within which comparatively short magnetic intervals may be, if not resolved, at least located with a fair degree of precision. This is the period of about 60 million years within the upper Carboniferous and Permian during which the geomagnetic field polarity was apparently almost always reversed. Until recently no normal intervals at all were known within this Kiaman reversed period (about 235 to 290 million years).

McElhinny (*Spec. Publs Geol. Soc. Austral.*, 2, 61; 1969), however, has reported a normal event at about 280 million years, which he named the Oak Creek event, and which Burek (*Bull. Amer. Assoc. Petrol. Geol.*, 54, 1120; 1970) later renamed the Graham event. Less well documented is a second normal event present in Italian mid-Permian sediments as reported by Guicherit (*Geologica Ultraiectina*, 14, 1; 1964). But the point is that normal events within the Kiaman reversed interval seem to be so rare that a few which may remain to be discovered are likely to be easily distinguished from each other and from those already found.

And so it has proved in at least one case, for Creer and his colleagues have discovered a new normal event well separated in time from the Graham event. The rocks in question are basalts, andesites and rhyolites of the Porphyritic Series exposed in Mendoza Province, Argentina. All samples were magnetically cleaned; and so there can be no doubt that normal polarities exist within this series. Moreover, the normal palaeomagnetic directions are consistent with the Permian field of South America as determined from the more common reversed rocks within the Kiaman interval. The stratigraphic age of the samples lies between about 270 and 210 million years; but, more importantly, the potassium-argon ages of two of the normal samples are 262 ± 6 and 264 ± 6 million years, respectively. The best age for the new normal event within the Kiaman is thus 263 ± 5 million years, which is distinguishable from the Oak Creek/Graham event.

PLANT ROOTS

Water Transport

from a Correspondent

A MOST useful exchange of information and ideas between plant scientists from both sides of the Iron Curtain took place from September 7 to 10 at Tatranská Lomnica, a mountain resort in the High Tatras National Park, during a symposium on the structure and function of primary root tissues. Unseasonably cool weather and showers lessened the lure of the mountains and the botanists were able to give their undivided attention to the matter in hand. The symposium was organized by the Institute of Botany, Slovak Academy of Sciences, and among the topics discussed were the organization and development of the root apex. Dr John Torrey (Harvard University) described some features of the quiescent centre—a group of non-dividing cells characteristically found in the middle of root apices—and speculated about its possible role in hormone biosynthesis.

Other sessions were devoted to consideration of the metabolic and enzyme basis of tissue differentiation and to the functioning of roots in the absorption and transport of water and mineral salts. One of the most stimulating and controversial contributions to the symposium programme concerned the absorption of water by root hairs. Dr Marcel Cailloux (University of Montreal) described an elegant technique which has enabled him to measure absorption of water by different parts of a single hair measuring less than 50 μm in diameter. Cailloux has observed that water is taken up much more readily by the growing tip of the hair than by the maturer regions and he associates this with an aggregation of cytoplasm at the tip. As growth of a root hair declines, so does its ability to absorb water and older hairs were found to excrete water rather than absorb it. These observations cast doubt on the commonly held view that root hairs serve to increase the area of absorbing surface in a root. They also raise the question of the mechanism of water absorption.

According to the classical view, the absorption of water by root hairs occurs by diffusion along a gradient of water potential between the soil solution and cell sap, which is maintained by accumulation of solutes. Cailloux disputes this because he finds that altering the relative humidity of the air surrounding the root hair from 99 per cent to 96 per cent, which is equivalent to an increase in the water potential gradient of about 44 bars, does not alter the absorption rate. Furthermore, metabolic inhibitors, such as malonate fluoride and cyanide, reduce absorption. It is concluded that water uptake by root hairs is controlled

by some process other than diffusion which is closely linked with metabolism.

The idea that water is "pumped" metabolically into plant cells is not a new one, but no such convincing evidence has been presented before. A major objection to the concept of active water transport has been the deduction that the resistance of cell membranes to flow of water by diffusion in either direction is so low that a water pump would be grossly inefficient and make impossibly high demands on the energy supplies of the cell. It remains to be seen whether the cell membranes of root hairs, and perhaps other specialized water absorbing cells in plants, have unique properties which enable these cells to pump water efficiently.

STEREOLOGY

Analysis of Structure

from a Correspondent

BERNE proved an excellent choice of venue for this year's meeting of the International Society for Stereology. The president of the society, Dr E. Weibel (Berne University), chaired the congress organization committee and the whole meeting, which took place between August 26 and 31, was run with Swiss precision.

The word "stereology" was coined a little more than ten years ago to cover the discipline of obtaining three-dimensional information from two-dimensional sections or their projections on a surface. There is now a wide interest in stereology, particularly among microscopists, and members attending this meeting—the third international congress—were drawn from such diverse fields as anatomy, materials science and mathematics.

The basics of stereology are obviously the mathematical relationships between

the object and the way it is imaged or sectioned. Professor G. Mathéron and his colleagues (Bureau de Recherches Géologiques et Minières, Paris) surveyed their work on the application of the theory of sets to this problem. A particularly interesting contribution from Dr R. Miles (Australian National University) derived one simple multi-dimensional formula from which all the standard formulae of stereology could be derived. Dr E. Underwood (Lockheed Georgia Research Laboratory, Atlanta), who is president-elect of the society, derived the formulae for the stereology of projected images and his approach was reinforced by Professor J. E. Hilliard (Northwestern University, Evanston) in a talk covering quantitative measurements on scanning electron micrographs.

Dr H. Elias (Chicago Medical School), the founder and first president of the society, illustrated the many pitfalls that could occur from a non-stereological interpretation of sections. Professor R. DeHoff (University of Florida) illustrated the use of serial sectioning in determining the topography of grains in metals and derived the basic equations of connectivity.

A major portion of the meeting was given over to the use of computers in image analysis with a survey from Dr G. A. Moore (National Bureau of Standards, Washington). It became apparent that the advent of cheaper computers of various types and interface software had already made stereology considerably easier for a much wider spectrum of scientists.

The generally held view was that this meeting was both excellently organized and scientifically worthwhile. It is quite clear that the International Society for Stereology will continue to grow rapidly and it is hoped that it will continue to maintain its interdisciplinary role.

Viscosity of the Earth's Core

IN next week's *Nature Physical Science*, Dr R. Hide of the Meteorological Office, Bracknell, argues that the kinematic viscosity of the Earth's core is not greater than $10^6 \text{ cm}^2 \text{ s}^{-1}$. Previous estimates of this parameter have varied between 10^{-3} and $10^9 \text{ cm}^2 \text{ s}^{-1}$ and Hide's new upper limit is a clear step towards pinpointing the viscosity more accurately.

Hide bases his arguments on the discovery of a statistically significant correlation between gross features of the Earth's gravitational and magnetic fields (see Hide and Malin, *Nature*, **225**, 605; 1970), and on variations in the length of the day, both of which suggest that the core-mantle boundary is not smooth but is characterized by

bumps which would seem to have horizontal dimensions of as much as a few thousand km and rather small heights of only a few km.

The height of a bump, Hide suggests, must be greater than the thickness of the viscous boundary layer at the core-mantle interface by at least a quantity determined by the horizontal dimensions of the bump, the Alfvén speed, the angular speed of the Earth's rotation and the radius of the core. Using a value of 1 km for the height of the bumps (as suggested by measurements of gravitational distortions) Hide deduces that the thickness of the viscous boundary layer must be $\leq 1 \text{ km}$ and, therefore, that the core viscosity is $\leq 10^6 \text{ cm}^2 \text{ s}^{-1}$.

CANCER CELLS

Chemical Vaccines

from our Cell Biology Correspondent

CANCER cells and cells transformed *in vitro* by chemicals or tumour viruses are about ten times more susceptible to agglutination by lectins such as concanavalin A or wheat germ agglutinin than are normal, untransformed cells. That much is generally agreed by those workers who have used lectins to investigate the surfaces of cancer cells. Why cancer cells are more agglutinable is of course still a matter of dispute; one camp argues that transformation leads to the exposure of previously cryptic lectin-binding sites whereas the other, which currently seems to have the upper hand, contends that it is the distribution of these sites rather than their number which differentiates the two classes of cells, the cancerous and the normal. But in any event there is a difference and it is common to all cancer cells, a fact which Shier has exploited in a remarkable set of experiments published in the current issue of the *Proceedings of the US National Academy of Sciences* (68, 2078; 1971).

Shier argues that because cancer cells are more readily agglutinable by wheat germ agglutinin than are normal cells (and he was assuming that this difference reflects a difference in the number of receptor sites exposed), it might be possible to immunize mice against tumours by raising an immune response against the wheat germ agglutinin receptor site. Exploiting Burger's discovery that this receptor is a soluble glycoprotein containing N-acetylglucosamine, he performed what seems to be an almost ridiculously simple and over-optimistic experiment, but the long shot has paid off handsomely.

Shier prepared a synthetic wheat germ agglutinin receptor site by condensing dimeric N-acetylglucosamine residues onto the sodium salt of polyaspartic acid and as a control he crossed linked polypeptide backbones without adding the N-acetylglucosamine residues. Having shown that wheat germ agglutinin has a much greater affinity for the synthetic receptor than for the control, the crosslinked polypeptide, Shier complexed the receptor and the control to methylated bovine serum albumin and used the two complexes (antigens A and B) to immunize mice in the presence of Freund's adjuvant. Two sets of mice, one immunized with the receptor-albumin complex and the other with the control antigen B, were then challenged with various numbers of cells of two BALB/c myeloma cell lines. Mice immunized with the synthetic receptor antigen rejected at least five-fold more tumour cells than those immunized with the control antigen B.

Furthermore, the time taken for palpable tumours to develop after exposure to a large dose of the chemical carcinogen 3-methyl cholanthrene was significantly greater after immunization with the receptor antigen than after immunization with the control. Mice which had been immunized with the receptor antigen grew normally and their cellular and humoral immune responses were not impaired although wound healing, as judged by liver regeneration, was slightly impaired compared with that of mice immunized with the control antigen.

In short, Shier seems to have raised an immune response not only against

transplanted tumour cells but also against the progression of cells transformed *in vivo* by a chemical carcinogen by immunizing against the wheat germ agglutinin receptor sites. No doubt hosts of laboratories will now be urgently mobilizing their chemists to prepare the antigens Shier has used and numerous variations on his theme, in attempts to confirm and extend his fascinating observations, which, although they defy simple interpretation, provide a most novel and promising lead to the pipe-dream of immunotherapy for cancer. It can only be hoped they are not too good to be true.

Atlantic Islands and Continental Drift

EVIDENCE has been accumulating that the Eastern Canary Islands (Fuerteventura, Lanzarote and Concepcion Bank) may play an important part in the fit of Africa and North America before continental drift. The most recent of such fits is by Dietz and Sproll (*Nature*, 226, 1043; 1970), who suggest that the most prominent gap (the Ioni Gap) is well matched by the East Canaries block. A small strike-slip translation of the East Canaries would essentially fill this gap. Dietz and Sproll's proposal that the East Canaries constitute a sialic continental fragment (a microcontinent) detached from the African margin is therefore attractive to geophysicists.

Before this idea is accepted, however, it is essential to take into account other evidence which might point to an origin for the Canary Islands (perhaps recent oceanic volcanism) fatal to the hypothesis. The bathymetry of this general region (from US Naval Oceanographic Charts) reveals that Fuerteventura, Lanzarote and Concepcion Bank together form a single elongate and largely submerged plateau lying sub-parallel to the African coast, with a submarine morphology not at all suggestive of a volcanic plateau. In contrast, the Western Canary Islands are revealed as rudely circular volcanic piles with much more steeply sloping sides than the East Canaries plateau. Seismic refraction and reflexion profiling, combined with gravity data (see, for example, Bosshard and Macfarlane, *J. Geophys. Res.*, 75, 4901; 1970), suggests strongly that the four most westerly Canary Islands do not form part of the African continent but are independent volcanic edifices lying on essentially oceanic crust. The geophysical work shows Gran Canaria to lie in a transitional area between oceanic and continental crust, but is at present inadequate for defining the crustal structure around Fuerteventura and Lanzarote, although it suggests that it is essentially continental.

The purely geological evidence bearing on this question is the subject of much controversy. The occurrence of carbonatites on the Eastern Canaries (but not on the Western Canaries) might be thought to constitute further evidence in favour of their continental nature, because recent surveys of carbonatite occurrences (*Carbonatites*, edited by Tuttle and Gittins, Interscience, 1966) show that they are typically found in fracture or rift zones in old shield areas.

Carbonatites were in fact reported from Fuerteventura by Fuster *et al.* in 1968, and from the Cape Verde Islands (which also have a bearing on this question) in 1965 by Assuncao *et al.* But the distinction between hydrothermal carbonate rich rocks and carbonatites is sometimes difficult on petrological grounds alone. In next Monday's *Nature Physical Science*, Allegre *et al.* report the results of carbon, oxygen and strontium isotopic ratio studies of rocks from Fuerteventura and the Cape Verde Islands which show them unequivocally to be true carbonatites. Portuguese geologists (Assuncao *et al.* have already suggested that the occurrence of carbonatites in the Cape Verde Islands could be explained if these islands were situated in the Mesozoic Mid-Atlantic Rift Valley when the North American and African continents started to drift apart. This suggestion can now be applied also to the Canaries.

It is worth noting that further support for this idea can be derived from the pre-drift fit of Dietz and Sproll, which results in the Fuerteventura carbonatite occurrence being located in fairly close opposition to the Canadian occurrences in the provinces of Ontario and Quebec. Nevertheless, problems remain for the petrologist in that the host rock of the carbonatites in Fuerteventura is a mafic and ultramafic plutonic formation similar in most of its features to the basements occurring in the truly oceanic islands of La Palma and Gomera.

BOTANY

Endemism in Manchester

from a Correspondent

A CONFERENCE on the taxonomy and phytogeography of higher plants in relation to evolution, held at the University of Manchester from September 9 to 11, was organized for the Botanical Society of the British Isles, the Linnean Society and the International Organization of Plant Biosystematists by Professor D. H. Valentine (University of Manchester), who gave a short opening address.

Endemism was perhaps the most dominant theme which emerged, and several speakers related this phenomenon to past geological and climatic history. In the Aegean area, endemism is clearly a consequence of fragmentation of the land mass, and this was described in some detail by Dr W. Greuter (Jardin Botanique, Geneva) for Crete and Dr A. Strid (Lund University) for the Cyclades. In older islands such as the Canaries (Dr D. Bramwell, University of Reading) the proportion of endemics is higher and good examples are found at all taxonomic levels. Professor C. Favarger (University of Neuchâtel) provided an analysis which showed higher proportions of endemic species in the southern European mountain ranges than in the central mass of the Alps, as a result of the greater isolation in the former. Dr S. M. Walters (University of Cambridge) outlined the special case of the almost totally apomictic *Alchemilla*, many of whose agamospecies are narrow endemics yet show some ecotypic differentiation. Professor H. Lewis (University of California, Los Angeles) distinguished between recent endemics of California which have arisen as a result of hybridization and those which have resulted from other sorts of isolating barriers. In the West Indies (Dr B. Morley, National Botanic Gardens, Dublin) the pattern of endemism in *Columnea* varies between the Greater and Lesser Antilles because of differences in geological history and the distribution of bird pollinators between the two areas.

As might be expected, differences of opinion were expressed concerning the mechanisms which have given rise to disjunct distributions, although the diversity of ideas is no doubt to be partly explained by one of the examples chosen. These ranged from bipolar (Dr D. M. Moore, University of Reading) to amphi-Pacific (Professor H. Hara, Tokyo University) distributions. Professor J. K. Morton (University of Waterloo, Ontario) considered that the scattered distribution of montane species in West Africa can be explained by cool periods during which the montane

species extended to lowland areas and migrated freely. Professor O. T. Solbrig (Harvard University) described semi-desert areas of great overall similarity in Mexico and Argentina, and outlined the aims and methods of an international team who are attempting to shed light on such situations by defining as precisely as possible the characteristics and relationships of the taxa and conditions involved. Dr F. Rose (King's College, London) presented evidence to show that the territory immediately north of the Somme Valley of northern France has been a greater barrier to the northern migration of species into Britain than has the English Channel, and that a number of continental species in southern England probably arrived from the former area direct and not by way of the Pas de Calais.

Hybridization is playing a major part in the modern rapid evolution of Hawaiian taxa. Dr G. W. Gillett (University of California, Riverside) pointed out that in many cases hybridization does not seem to be related to recent human activity, because it occurs in wind as well as in insect-pollinated genera (and is hence not related to human effects on pollinators) and the open communities favoured by hybrid progenies and segregates are provided by natural phenomena such as lava flows. Professor H. G. Baker (University of California, Berkeley) expounded his ideas on weed evolution with reference to California and described many different methods by which plants had become successful weeds there. Native species are most likely to become weeds in man-made situations which mimic natural ones, whereas alien weeds more often colonize new sorts of habitats.

The migration of taxa was the theme of several other contributions. Professor G. L. Stebbins (University of California, Davis) discussed the notion that angiosperms had originated in mesic conditions and subsequently migrated to areas showing extremes of temperature and water availability. Such a theory is based on the belief that the major characteristics of taxa and their evolution are similar now to when the angiosperms arose. Professor C. van Steenis (Rijksherbarium, Leiden) described the "hour-glass" type of world distribution in which there are wide temperate ranges linked by a narrow tract through Malaysia. Such situations are often accompanied by vicariousness at the family, genus or species level, for example, *Fagus* and *Nothofagus*. *Nothofagus* is now southern temperate, but probably arose further north and migrated to the south by way of Malaysia. A similar route was postulated for *Epilobium* by Professor P. H. Raven (Missouri Botanic

Garden), because the genus is in many ways more stereotyped in Australasia than in the northern hemisphere even though there are more species compared with the area covered. Professor T. W. Böcher (University of Copenhagen), on the other hand, discussed east-west migrations in the Arctic region.

Inter-continental similarities and differences have often been investigated by anatomical means, and two contributions exemplified different facets of this sort of study. Dr D. F. Cutler (Royal Botanic Gardens, Kew) showed how a superficially similar stem anatomy in the Restionaceae is accomplished by quite different developments, and Dr A. W. Exell (British Museum (Natural History)) and Dr C. A. Stace (University of Manchester) described situations in the Combretaceae where certain apparently non-adaptive features were limited to one continent or another throughout a wide taxonomic spectrum.

MYCOLOGY

International Liaison

from a Correspondent

AT the First International Mycological Congress held at the University of Exeter from September 8 to 14, an International Mycological Association was established for the encouragement of mycology in all its branches, particularly international aspects, by promoting international mycological congresses and by liaison with other relevant international and national bodies. Membership of the association is open both to international and national societies or groups having mycological interests and to individual mycologists in countries lacking a mycological society.

According to the *International Mycological Directory* (published for the congress by the Commonwealth Mycological Institute, Kew, Surrey, and available at £0.50, post free), there are already a dozen series of international meetings devoted to special branches of mycology, and there are at least an equal number of recurring international congresses or meetings which include some mycology. An important task of the new association will therefore be to attempt to rationalize these events.

The first officers of the association are: president, Professor C. J. Alexopoulos (USA); treasurer, Dr J. A. von Arx (Netherlands); secretary, Dr C. Booth (Commonwealth Mycological Institute, Kew, Surrey, England).

Immunoglobulin E (Reagin) and Allergy

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A tissue-sensitizing factor found in the sera of allergic subjects has been identified as a unique immunoglobulin, IgE. Investigations of its mode of action could lead to the development of new forms of treatment.

FIFTY years ago Prausnitz showed that it was possible to sensitize sites on his forearms by the intradermal injection of serum from one of his allergic patients (Küstner) who was hypersensitive to fish¹. The injection of fish extract into the same sites 24 h later evoked immediate wheal and erythema reactions similar to those shown by the patient himself (and by other fish-sensitive individuals) in response to the direct intradermal injection of fish extract.

This observation was a historic "landmark" in a field which has not always been noted for objectivity. Besides demonstrating that human hypersensitivity of this immediate-type could be transferred to normal tissue, outside the influence of the allergic individual, it meant that a passively induced reaction could be restricted to a localized area of the skin with minimal discomfort to the non-allergic recipient. Of even greater significance, however, was the inference that immediate hypersensitivity (unlike that of the delayed type, of which tuberculin sensitivity is an example) was mediated by a humoral factor.

There soon followed similar demonstrations of the local transfer of human hypersensitivity to many common inhalants (such as grass pollens, animal danders and house dusts); the Prausnitz-Küstner (P-K) test, as it is known, being used as an alternative to direct skin testing in the diagnosis of hay fever and allergic asthma. Its principal application, however, has been in the experimental study of immediate-type hypersensitivity; where, until a few years ago, it offered the only satisfactory means of assay. Consequently attempts to characterize the skin sensitizing factor more fully have been seriously handicapped.

Early investigations showed this serum constituent to resemble antibodies which appear in the circulation of humans and animals in response to conventional immunization procedures. But it seemed to lack certain properties commonly associated with immune antibodies, such as the capacity to form a precipitate and to fix complement when combined with specific antigens *in vitro*. For these reasons, some investigators² preferred to reserve judgment on its antibody status by terming it "atopic reagin" ("atopy" denoting a form of human hypersensitivity in which there was evidence of a hereditary predisposition, the sensitizing substances being referred to as "atopen"). The factor demonstrable in the serum of individuals with immediate hypersensitivity of the asthma-hay fever-urticaria type, has since been termed "reagin" and it has been customary to refer to the provoking agents (in inhalants, foods and so on) as "allergens".

P-K testing revealed that reagins become firmly "fixed" in normal isologous human skin where they are detectable (by subsequent allergen challenge) several weeks after the transfer of the allergic serum. In contrast, antibodies produced by the immunization of rabbits with allergenic substances (such as pollen extracts and egg protein) failed to fix to human skin;

although unlike reagins, they could passively sensitize the skin of heterologous guinea-pigs for relatively short times. Human antibodies (γ G type) against diphtheria toxoid likewise disappear rapidly when injected into isologous human skin (with a half-life of 12 h)³, whereas reagins transferred isologously have been estimated⁴ to have a "half-life" of 15 days.

Quantitative P-K testing⁵ based on the accurate measurement of the areas of wheals produced on the backs of normal recipients showed that a maximal skin response was achieved with a time interval of about 50 h between transfer of allergic serum and challenge with specific allergen. Once this was effected, however, a wheal and erythema reaction developed rapidly with the wheal approximately doubling in area during 10–20 min after introduction of the allergen. The susceptibility of reagins to relatively mild heat treatment was also confirmed⁵. Similar conditions are used in the destruction of the complement activity of immune sera, but the addition of fresh normal human serum to heated allergic serum fails to restore P-K activity⁶ and other evidence indicates that reagins do not depend on the complement system for their activity.

Reagins were also found to differ from immune (7S type) antibodies in their inability to cross the placenta. This situation is obviously fortunate for the offspring of allergic women, as is the apparent absence of reagins from human colostrum. It has been attributed by some investigators to the macroglobulin (19S) antibody nature of the tissue sensitizing factor, which was thought to be of sufficiently large molecular size to be retained by the human placenta's supposed sieving mechanism. But extensive studies by Brambell and his associates⁷, and others, have indicated that the placental transmission of immunoglobulins is a highly selective process (controlled by sites located within their Fc regions).

The biological properties of reagins revealed by these early investigations, based principally on P-K testing, are summarized in Table 1. This was the position around 1940, when techniques of free-solution electrophoresis and ultracentrifugation were first applied to the fractionation of plasma proteins. Although such physico-chemical procedures offered better means of separating complex protein mixtures than had been previously possible, by salt or low temperature-ethanol precipitation, they were essentially analytical tools. Nevertheless, electrophoretic analyses of the sera of animals before and after immunization provided the first evidence of the γ globulin nature of precipitating antibodies⁸; while corresponding ultracentrifugal studies indicated a size heterogeneity (antibody activity being associated with both 7S and 19S components).

It was naturally hoped that a similar approach would provide more convincing evidence of the antibody nature of reagins; but this was thwarted by practical difficulties. It was established, however, that reagins were electrophoretically faster than immune γ globulins, moving in the β region (where

Table 1 Main Biological and Physico-chemical Properties of Reagins (γ E-Antibodies)

- (1) Bind firmly to isologous, and closely related heterologous, tissues
- (2) Heat labile (destroyed by 56° C for 1 h)
- (3) Not dependent on complement
- (4) Fail to cross the placenta
- (5) Move in the "fast γ " region on electrophoresis
- (6) Sediment near to 8 S

19S type immune antibodies were also found). But this conclusion was very much an extrapolation, and the minute amount of skin sensitizing factor present in allergic sera could have been moving in the electric field in combination with a major constituent. More refined preparative fractionation procedures were required.

Physico-chemical Characteristics

As in the characterization of many other minor active protein constituents of biological fluids, it was the advent of zone-fractionation procedures which gave the first definitive indication of the physico-chemical characteristics of the skin sensitizing factor. Thus, zone electrophoresis of allergic sera in starch blocks⁹ confirmed that reagins moved in the "fast γ " (slow β) region; whilst zone-centrifugation in buffered sucrose gradients¹⁰ showed clearly that they sedimented in the 7S region (and were not macroglobulins, as had been suggested to explain their failure to cross the placenta). There were, of course, limitations to this type of correlative approach because each of the major serum electrophoretic fractions, including the γ fraction, comprised several components. It was at this stage, however, that the application of highly-

specific and sensitive immunological techniques began to compensate for the shortcomings of the physico-chemical procedures. Studies on the monoclonal γ globulins, isolated in large quantities from the sera of patients with multiple myelomatosis and Waldenström macroglobulinaemia, led to the production of specific antisera which distinguished three major immunoglobulin classes¹¹: γ G or IgG (previously referred to as 7S γ globulin); γ M or IgM (previously referred to as 19S macroglobulin) and γ A or IgA (a newly discovered third class¹², which when isolated from normal human serum by a zinc precipitation procedure was found to comprise a major 7S component, with minor amounts of polymerized material, and which migrated in the fast γ region on electrophoresis). It was established that the three major immunoglobulin classes then known possessed common light polypeptide chains, but distinctive heavy chains (Fig. 1).

The problem then was to show that skin sensitizing activity was a property of one (or perhaps more) of these antigenically distinguishable immunoglobulins. Antigenic and physico-chemical analysis of fractions of allergic human serum separated by chromatography on DEAE-cellulose (which provided a high degree of resolution of the various classes of immunoglobulin) revealed maximal P-K activity in a fraction in which

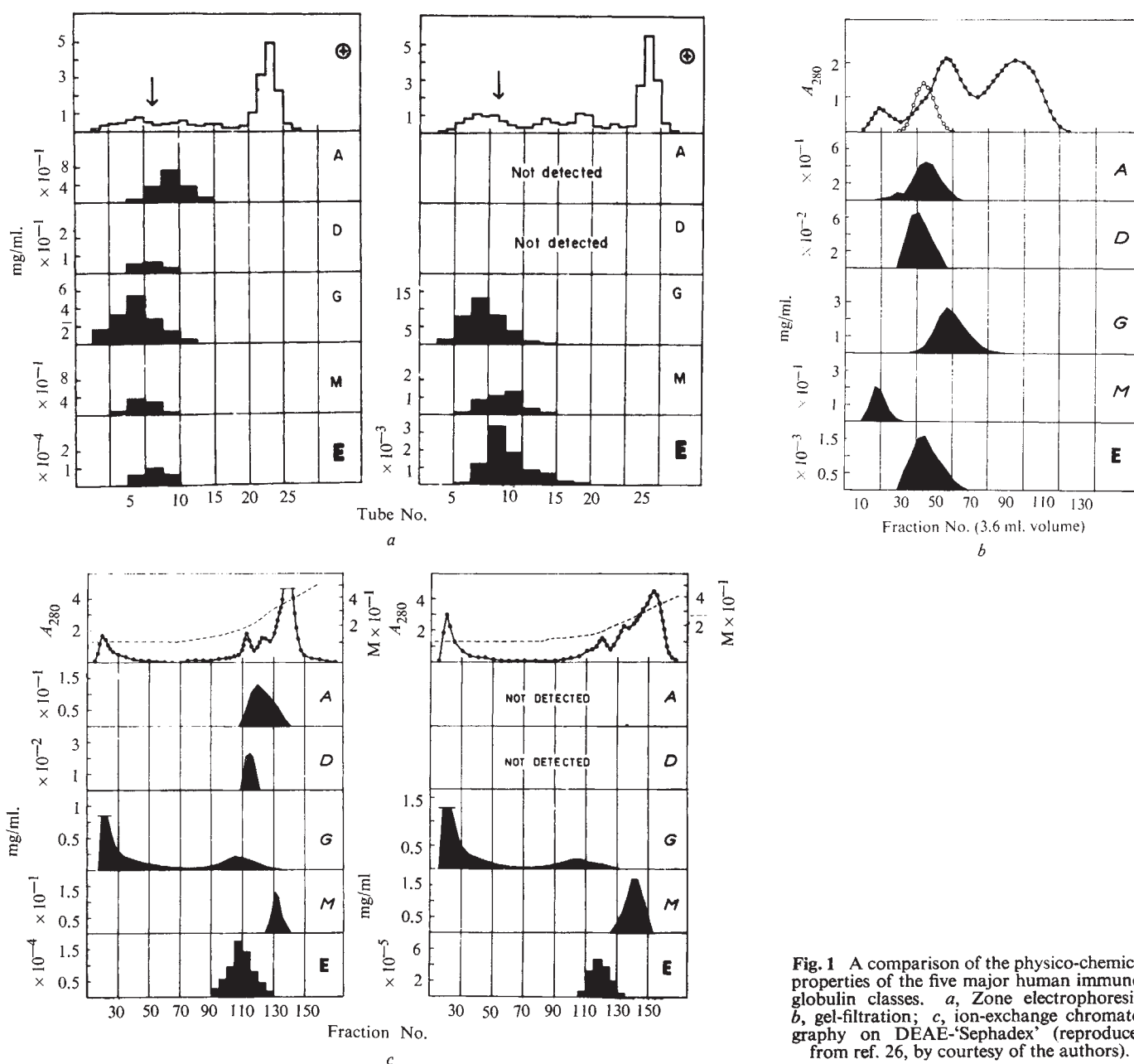


Fig. 1 A comparison of the physico-chemical properties of the five major human immunoglobulin classes. *a*, Zone electrophoresis; *b*, gel-filtration; *c*, ion-exchange chromatography on DEAE-Sephadex (reproduced from ref. 26, by courtesy of the authors).

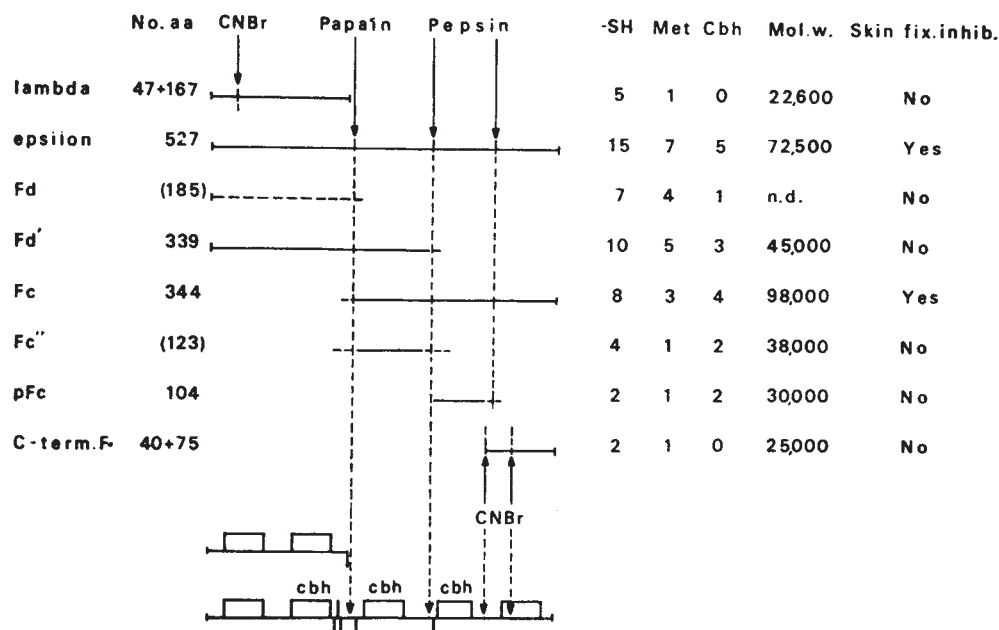


Fig. 2 Working model for molecular structure of IgE indicating the disposition of antigenically and biologically active fragments, and carbohydrate (cbh) prosthetic groups. (Reproduced from ref. 62, by permission of the authors.)

only electrophoretically fast 7S γ G globulin could be detected. As Fig. 2 shows, this was in an elution position well ahead of any detectable γ M globulin; and even ahead of any γ A globulin detectable by immunodiffusion analysis using specific antisera¹³.

This latter observation was particularly significant, at a time when many claims were being made that reagins were γ A globulins. These were based on such evidence as the recovery of P-K activity in highly purified γ A globulin preparations¹⁴, the removal of activity from allergic sera by absorption with specific sheep anti-human γ A globulin antiserum¹⁵ and the blockage of P-K reactions by normal human γ A globulin or its isolated heavy chains¹⁶. They were of obvious teleological appeal as γ A globulin had been found in high levels in nasal and other secretions, where it supposedly acted as a "front-line" defensive agent. But a lack of correlation was observed between reagin activity and γ A globulin distribution in DEAE cellulose chromatographic fractions of allergic sera¹³; and other studies¹⁷ suggested that P-K activity was associated with a minor component of the sera of hypersensitive individuals, which had appeared as a contaminant in the supposedly pure γ A globulin fractions investigated previously.

Were reagins a unique type of immunoglobulin (a prospect on which I had speculated in 1963 (ref. 18))? The discovery of a new human immunoglobulin class (IgD or γ D)¹⁹, which resembled γ A globulin electrophoretically, seemed a possibility; but several independent investigations suggested this immunoglobulin did not possess skin sensitizing activity.

Reagins were found, however, to have unusual physico-chemical characteristics as well as distinctive biological properties (for antibodies). For example, the gel filtration of sera of people sensitive to ragweed (on 'Sephadex G200'²⁰) led to the recovery of maximal P-K activity slightly ahead of the 7S peak containing the γ G and γ A globulins, but well behind the 19S peak containing the γ M globulin. Furthermore,

reagins in such sera were found by density gradient ultracentrifugation²¹ to possess a sedimentation coefficient of 7.7S (7.4–7.9S range)—significantly greater than human γ G and γ A globulins. It also seemed significant that the reagin-like antibodies, occurring naturally in some animals (such as the dog) and induced artificially in others (for example, rats and rabbits), had sedimentation coefficients greater than 7S but much less than 19S (Table 2).

Further evidence that reagins were probably a unique class of immunoglobulins was obtained by Ishizaka and his associates²², who isolated active preparations by application of a multi-step procedure to the fractionation of pools of sera from individuals showing marked hypersensitivity to ragweed pollen and who developed highly sensitive radioimmune-diffusion assays as *in vitro* alternatives to the P-K test for the measurement of the reagin in the isolated fractions. Reagin preparations thus obtained were used to produce anti-reagin antisera, which were rendered specific by absorption with the major classes of immunoglobulin and which were used in radio-immunoelectrophoresis to provide evidence that a radio-labelled ragweed allergen (E) preparation combined with the reagin isolated from ragweed-sensitive individuals' sera.

Ishizaka and associates²³ suggested that reagin activity was carried by a unique immunoglobulin which they tentatively termed " γ E" (in view of its specific binding capacity for ragweed allergen E). Despite the evidence presented in support of this idea, however, some investigators felt that such claims were perhaps a little premature until chemical evidence was available to suggest the existence of a unique type of polypeptide chain.

Myeloma Protein

The final evidence that reagins were a unique class of immunoglobulins came with the isolation of an atypical myeloma protein (originally designated "IgND"), from the serum of a patient ("ND") with myelomatosis and Bence Jones proteinuria²⁴, which lacked the antigenic determinants characteristic of the heavy polypeptide chains of the then known immunoglobulin classes (γ G, γ M, γ A and γ D) while possessing similar light chain determinants (of sub-type L). Like myeloma γ A and γ D globulins, this atypical protein moved in the "fast γ " region on zone electrophoresis (Fig. 1); but it was eluted slightly ahead of these classes of immunoglobulin during chromatography on DEAE-'Sephadex'. Of greater significance, however, was its elution position during gel-filtration on 'Sephadex G100', where it emerged after the γ A globulin but just ahead of the γ G globulin. Furthermore, free solution

Table 2 Sedimentation Characteristics of Reagin-type Antibodies formed in Various Mammalian Species (as determined by Density Gradient Ultracentrifugation)

Species	Mode of antibody formation	Sed. coeff. (S°)
Human	Spontaneously	> 7.4 S–7.9 S
Dog	Spontaneously	6.8 S–10.1 S
Rat	Experimentally	> 7 S, < 19 S
Rabbit	Experimentally	> 7 S, < 19 S

ultracentrifugal analysis²⁵ showed the atypical myeloma protein (IgND) to possess a sedimentation coefficient ($S_{20,W}$) of 7.92S (and molecular weight 196,000 from Archibald analysis); a value very similar to that which had been assigned to the reagins in sera of ragweed-sensitive individuals on the basis of the observed rate of sedimentation of the P-K activity in buffered sucrose gradients.

This similarity in sedimentation coefficient between the new type of myeloma protein and reagin could have been fortuitous, but it seemed significant that the myeloma protein isolated from the serum of patient "ND" was the first known type of immunoglobulin to have similar size characteristics to the skin sensitizing factor. Other observations were beginning to suggest a relationship between the new class of immunoglobulin and reagin. For example, by means of a radio-immunosorbent technique based on the use of 'Sephadex'-coupled antibody directed specifically against the new myeloma protein²⁴, a significantly elevated concentration (5,900 ng/ml.) of an antigenically similar protein was detected in the serum of an individual with allergy to dog dander; in comparison with the much smaller amounts (100–700 ng/ml.) found in normal sera. Later comparative studies²⁶ of the antigenic characteristics of the new myeloma globulin and the reagin-rich fraction (designated γE) isolated from sera of ragweed-sensitive individuals were to provide evidence of a close structural relationship between the two proteins.

The most convincing evidence that the myeloma protein (IgND) was a pathological counterpart of reagin was obtained, however, from inhibition-P-K testing in a normal human recipient²⁷. The intradermal injection of the myeloma protein mixed with, or 24 h before, the sensitizing serum (at a dosage of 5–50 times in excess of the level of reagin in the serum) completely inhibited a weal and erythema response on subsequent challenge with the specific allergen (isolated from horse dandruff). Thus not only was the myeloma protein (IgND) antigenically similar to reagin, it also seemed to have its striking affinity for isologous tissues. There was no evidence, however, that the myeloma protein could bind antigen (that is allergen). Thus it seemed to be a pathological counterpart of a new immunoglobulin class of which reagin was a normal representative, just as an over-production of monoclonal forms of the major immunoglobulin classes has often been seen in plasmocytic and lymphocytic neoplastic disorders.

Immunological and chemical studies²⁵ indicated that the myeloma protein IgND was a new class of immunoglobulin, with similar light chains to those in the other four classes, but distinctive heavy polypeptide chains somewhat larger (75,500 molecular weight) than those in the other immunoglobulins. Consequently at a meeting initiated by the World Health Organization in Lausanne in 1968 (ref. 28) it was decided that there was sufficient evidence to conclude that reagin was representative of this new class of immunoglobulin, which it was proposed should be designated "IgE" or " γE ".

The availability of the myeloma protein offers a means of chemically characterizing reagins, and ultimately of explaining their unusual biological properties in structural terms. It has been calculated that the chance of finding a case of monoclonal production of γ globulin of the E type is about one in 50,000 that of finding a more common case of myelomatosis; so the protein isolated from the serum of the Swedish case has proved particularly valuable and highly sought after. Fortunately, however, a second case has been reported²⁹ in the United States, who has clinical manifestations similar to those seen in the case originally reported by Johansson and Bennich in Sweden.

Already the two γE myeloma proteins are providing important information about the role of the antibody (reagin) in immediate hypersensitivity reactions. For example, the myeloma IgE has been degraded into different types of polypeptide fragment by means of proteolytic and chemical cleavage procedures, and tested the ability of these to inhibit skin sensitization by the reagins (γE antibodies) in the sera of

individuals sensitive to horse dandruff and grass pollen^{30,31}. Of the various types of fragments tested (originating from the different parts of the IgE molecule as illustrated schematically in Fig. 2) only those incorporating the Fc region had the inhibitory activity of the parent molecule. Similar findings have resulted from inhibition studies in sub-human primates (for example, rhesus monkeys and baboons) which, like non-allergic humans, have proved receptive to sensitization by human γE antibodies but which (for obvious reasons) are now preferred as test recipients.

Other inhibition sensitization tests in baboons³², have involved myeloma IgE preparations which have been reduced to various extents with 2-mercaptoethanol, to ascertain the importance of the relatively large number of disulphide bridges in maintaining the conformational integrity of that part of the IgE molecule involved in tissue binding.

Binding of IgE

Inhibition tests with proteolytic cleavage fragments of myeloma IgE have led to important conclusions about the binding of antibody IgE to isologous (or closely related heterologous) tissue. They suggest that, like the myeloma protein molecules, the antibody (reagin) molecules similarly bind to the tissue receptors through sites within their Fc regions (Fig. 3). Sites within the Fab region of the cell-bound antibody molecules are thus free to react subsequently with antigen (allergen) in the usual manner. In contrast, the myeloma IgE molecules (like most myeloma forms of the other immunoglobulin classes) cannot combine with antigen (Fig. 3). If, on the other hand, combination with the target cells had been found to occur through the Fab regions of the antibody IgE molecule, it would have been necessary to infer that immediate-type hypersensitivity is an auto-immune phenom-

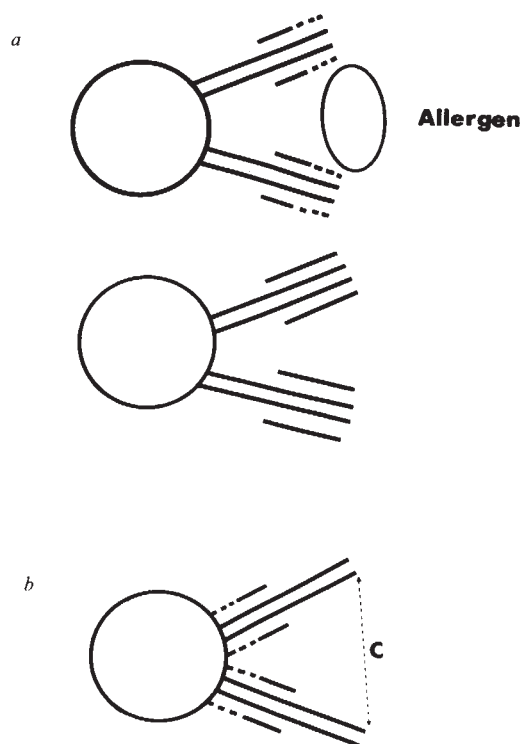


Fig. 3 Diagrammatic comparison of the properties of antibody and myeloma γE molecules, showing mode of attachment to target cells (that is by means of sites in the Fc regions). *b*, Mode of interaction of γG type cytolytic antibody molecules with target cells, showing subsequent interaction of complement (*c'*) with sites located in the Fc regions.

enon involving the production of antibody against self-cell surface antigen (as occurs, for example, in autoimmune haemolytic anaemia).

Thus it is now possible to start explaining how the immunological reactions occurring at tissue mast cell surfaces bring about the pharmacological manifestations characteristic of hypersensitivity reactions of the immediate-type. Whether such reactions are mediated by γ E-type antibodies in passively sensitized human tissues (as, for example, in the classical P-K test), or by non-reaginic γ G-type antibodies in the heterologous tissues of guinea-pigs passively sensitized with the serum of experimentally immunized animals, the sequence of events is remarkably similar. The antibody plays a crucial role, not only in the initial sensitization process (which, of course, occurs spontaneously in the allergic individual or the actively sensitized guinea-pig), but also in the manner in which it subsequently interacts with specific antigen (allergen) to activate the enzyme system supposedly responsible for effecting the release of histamine, 5-hydroxytryptamine and other pharmacologically active substances. The elucidation of the structural basis of these different, but complementary, functions of tissue-sensitizing antibodies poses a problem of protein characterization. Moreover, it seems likely that studies along these lines on γ E globulins will throw light on the mode of action of other classes of immunoglobulin fulfilling other roles on the surfaces of other types of cell (for example, receptor immunoglobulin or lymphocytes).

The emergence of *in vitro* alternatives to the P-K test in humans, and the PCA test in sub-human primates, is facilitating the delineation of the biochemical events set in train by the combination of cell-bound γ E antibody and allergen. Such systems are based on passive sensitization by γ E antibodies of chopped human lung³³, normal human leucocytes³⁴, chopped monkey skin³⁵ or other suitable primate tissue. The histamine released from the washed sensitized cells or tissues as a result of interaction with specific allergen can be measured accurately (down to levels of a few nanograms) by bioassay using isolated guinea-pig ileum, but a spectrofluorometric procedure involving coupling with ophthalaldehyde³⁶ has been adopted as a satisfactory chemical alternative. These indirect procedures have obvious advantages over the previous pharmacological approach to the study of immediate hypersensitivity reactions, which involved the direct assay *in situ* of the histamine liberated on presentation of the specific antigen to the actively sensitized tissue set up in an organ bath. Their application has provided convincing evidence^{37,38} that the allergen-induced release of histamine and other mediations of immediate-type hypersensitivity reactions is accomplished by a multi-step, energy-requiring process to which the glycolytic pathway is essential. There are precise pH and temperature requirements, and a dependence on calcium and magnesium ions.

It is important, however, to distinguish this process from cytolytic reactions in which destruction of the cell membrane is effected through the combined agency of anti-cell antibody and complement. In contrast, γ E-mediated histamine release has been shown (from K^+ efflux studies³⁸) to be an active secretory process which is not intrinsically injurious to the target cell. Thus, there is a fundamental difference in the release mechanism initiated by sensitizing antibodies (for example of the γ E type), in the absence of complement, and those more drastic processes induced by cytolytic antibodies (for example of the γ G type) with the aid of the complement system. As I have already implied (Fig. 3), the type of process evoked depends on the manner in which the antibody is presented to the cell. Antibodies of the γ E type seem particularly suited to binding to histamine-containing cells through sites on their Fc regions, and to possess within their own structures sites capable of direct action on the target cell membrane (or other cell constituents). On the other hand, cytolytic antibodies (whether directed against cell membrane antigen, or coating antigen) react initially through sites within their Fab regions, a subsequent intermolecular interaction between their free Fc

regions³⁹ supposedly leading to the activation of the complement system responsible for the ensuing lysis.

Tertiary and Quaternary Structure

The problem now is to establish how the interaction of the cell-bound γ E antibody (reagin) with allergen triggers off the events outlined, which seem (from microscopic studies of viable cell preparations) to be associated with characteristic morphological changes⁴⁰ in the target cells, involving the release of their histamine-containing granules. The amino-acid sequencing in progress on the two myeloma IgE preparations should provide important information as might the characterization of the relatively large carbohydrate (11.7%) prosthetic group. Furthermore, studies of the inhibitory capacity of proteolytic cleavage fragments of the myeloma IgE (mentioned earlier) are being extended to smaller and smaller fragments, with the hope of isolating a readily characterizable low molecular weight peptide retaining tissue-binding activity. It is becoming increasingly obvious, however, that the peculiar biological properties of γ E antibodies (as well as those of the less efficient γ G type sensitizing antibodies produced in experimental animals) will be explicable in terms of unusual tertiary and quaternary structural characteristics. It seems likely (as discussed fully elsewhere⁴¹) that the two structural features which will prove to distinguish sensitizing antibodies will be an ability to bind strongly to complementary tissue sites with no accompanying loss in the conformational integrity of their Fc region (which can soon occur as a result, for example, of relatively mild heat treatment); and the possession of sufficient flexibility within the "hinge regions" to permit the transmission of a critical allosteric change resulting from combination of allergen with sites in the Fab regions of adjacent cell-bound molecules.

There is evidence⁴² that antigen-induced conformational changes can occur in free solution, in rabbit antibody directed against commonly used antigens such as bovine serum albumin and horse ferritin, and these lead to the exposure of new antigenic determinants within the Fc regions. It seems reasonable to conclude, therefore, that similar conformational changes could occur in γ E-type antibody molecules in combination with specific allergen (as already proposed⁴³). Moreover, if the γ E antibody is already anchored to the target cell surfaces, through sites within its Fc region, newly exposed side chains could be brought into critical juxtaposition with points on the cell where activation of the appropriate enzyme system would occur (Fig. 4). Indeed there is evidence (from inhibition studies with aggregated IgE) that association of IgE molecules might lead to the formation of "tissue activation sites" which are distinct from the tissue binding sites in monomeric (antibody and myeloma) IgE molecules; and it is possible that activation sites are similarly formed in the Fc regions of adjacent cell-bound sensitizing antibody molecules as a result of the bridging by specific allergen, for which there is increasing evidence^{45,46} and which presumably involves the cross-linking of sites within the antibody Fab regions (Fig. 4). The critical question then to be answered is whether groups exposed from the Fc regions of the cross-linked antibody molecules act cooperatively to form the activation site. In this event, the activation site could be linked to the active site of an enzyme, which of course is usually contained within a single polypeptide chain. Alternatively, the side-chains which are supposedly exposed in the Fc regions of the sensitizing antibody molecules might be proved to exert an independent effect on the target cell.

In attempting to decide between these alternatives it is worth considering the results of studies designed to short-circuit the histamine release process initiated by reagin-allergen combination. These involve the use of alternative, artificial, ways of cross-linking the γ E molecules, and seem to lead to pharmacological responses similar to those of classical immediate sensitivity reactions. Apart from the pre-aggregation

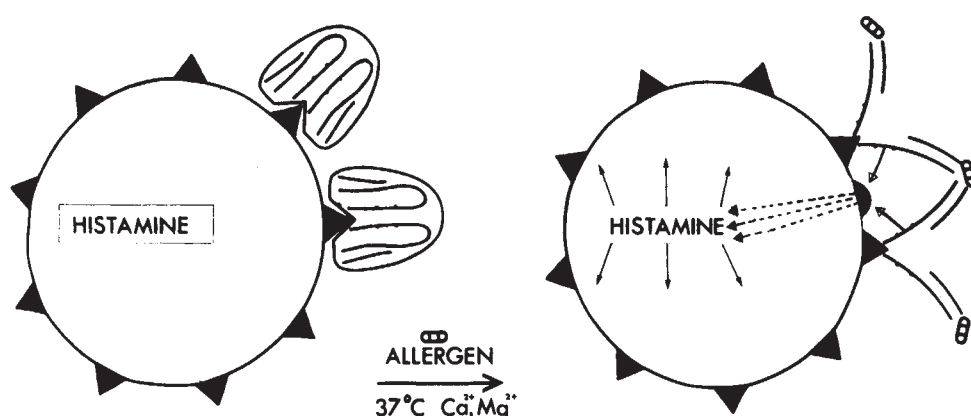


Fig. 4 Postulated mode of interaction between cell-bound γE antibodies and allergen leading to the triggering of release of vasoactive amines (modified from ref. 45).

of the γE molecule before presentation to the target cell (as already mentioned), it is possible to effect histamine release in human or monkey skin by bringing about the cross-linking of the cell-bound γE molecules with antibody directed specifically against determinants within their Fc regions. Thus, it can be demonstrated⁴⁶ that the intradermal injection of specific anti-human IgE antiserum induces an immediate oedematous and erythematous reaction similar to that observed in the P-K test.

Tissue Activation Sites

It is suggested that, as in the classical reagin-allergen mediated reaction, the exposure of tissue activation sites within the Fc region of the γE molecule triggers the release of the pharmacologically active mediators. But the cross-linking process which induces the required conformational change is less efficient than that effected by the cross-linking of the Fab regions of the γE antibody molecules by allergen. This might be expected by reference to the model shown in Fig. 5, which suggests that a force applied at position A (at maximum distance from the fulcrum) would be the most effective way of forming the activation site at position S; but application of a force at position B would accomplish the same effect in a less efficient manner. This analogy could offer an explanation of the skin reactions effected by the action of anti-IgE antibody, as well as that elicited by other agents such as protein A (an antigenic component of *Staphylococcus aureus*) which has an unusual capacity for combination with the Fc regions of immunoglobulins⁴⁷ and which presumably acts too by cross-linking cell-bound γE molecules⁴⁸.

Alternatively, it seems likely that the conformational changes necessary for the formation of tissue activation sites can also occur in non-specifically aggregated IgE (brought about, for example, by lyophilization of monomeric myeloma IgE). As

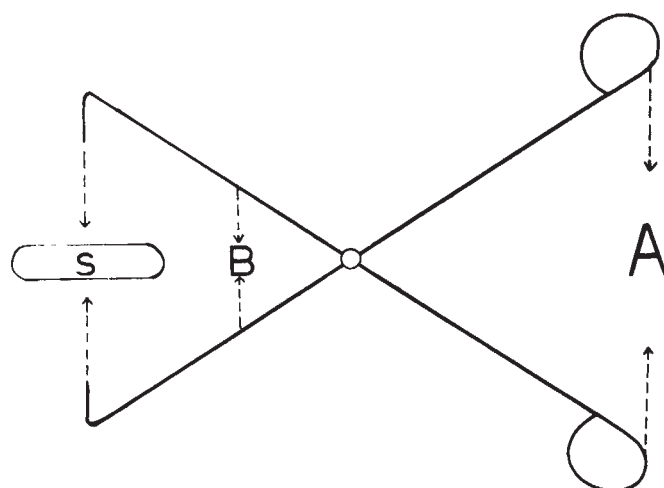


Fig. 5 Model illustrating various methods of inducing an allosteric transformation within cell-bound γE antibody molecules.

already mentioned, IgE treated in this way is also capable of effecting an immediate release of histamine on injection into human or monkey skin⁴⁶. Thus, perhaps surprisingly, in spite of the non-specific nature of the aggregation process, a conformational change seems to be induced within the Fc regions similar to that resulting from antigen-mediated association of the γE molecules. This is directly analogous, however, to the situation with regard to the association of human (and rabbit) γG molecules where biological activities (for example heterologous skin reactivity and complement fixability), and new antigenic determinants (similar to those formed on specific antigen-antibody combination) are induced by non-specific denaturation treatments. Moreover, recent studies⁴⁹ on the nature of the structural changes shown by γG globulins as a result of physical or chemical treatments suggest that here too association is initiated by preliminary (non-covalent) interaction between the Fab regions of the molecule but in this case, of course, without the agency of the antigen. This, it seems, leads to an unfolding of the Fc regions, similar to that occurring in antibody γG molecules on combination with antigen, thereby providing scope for intermolecular Fc interaction with formation of the sites of the various biological activities shown by aggregated γG globulins. Thus what might be expected to be a random aggregation process seems to be a highly ordered polymerization, directed presumably by the initial intermolecular reactions of the Fab regions of the monomer molecules.

The complete characterization of these crucial tertiary and quaternary structural changes could have an important bearing on the explanation of other immunological phenomena. As far as immediate hypersensitivity is concerned, the need now is to characterize the "tissue activation sites" formed in γE globulin molecules on association, and to elucidate the nature of their "substrate" located on the target cells. Various possibilities are being considered. For example, could it be that reagin-allergen combination on the cell surface activates a phospholipase responsible for hydrolysis of membrane phospholipid⁵⁰; or perhaps an esterase is activated, in a similar manner to that implicated in complement mediated cytolytic reactions⁵¹. Another possibility⁵² is the stimulation of the adenylyl-cyclase system, which is located on the inner surface of cell membranes (where it catalyses the conversion of ATP to cyclic AMP) and which has been shown to be involved in the action of certain hormones⁵³. It could be more than fortuitous that certain polypeptide hormones (such as ACTH) which act in this manner are also potent histamine liberators⁵⁴. It will, however, be important to exclude the simpler possibility that the primary triggering mechanism is a physical shearing of the cell membrane ("rigidification", as one investigator⁴⁴ has termed it), brought about by the association of surface-bound γE antibody molecules.

Future Tasks

Although the initiative is now passing to biochemists and biophysicists, many other aspects of the behaviour of γE anti-

bodies require immunological investigation. For example, with the aid of specific antisera prepared against the myeloma proteins, it is possible to begin to identify the sites of binding of γ E antibodies on their target cells; as well as their sites of synthesis in human lymphoid tissues. Immunofluorescence studies⁵⁵ have provided evidence that γ E antibodies are formed locally in the respiratory and gastro-intestinal tract where they probably become involved in allergic states affecting these organs. In any studies of γ E antibody synthesis it will be important to answer the fundamental question about the definition of the factors which govern the production of sensitizing rather than immunizing antibodies. Here the use of animal models⁵⁶ is particularly revealing, because it is becoming apparent that the reagin-like antibodies produced in species like the rat, the rabbit and the mouse closely resemble the human γ E antibodies. The chemical nature of the allergen, and its form and mode of administration are proving important factors in deciding the nature of the resultant antibody response, as is the influence of other classes of antibody produced concomitantly. Through such investigations it is becoming possible to define the immunological basis of "desensitization" (that is hyposensitization), a prophylactic measure which has been practised by clinical allergists for several years and which is thought to encourage the production of γ G-type antibodies which bind allergen preferentially (besides producing effects at the cellular level).

Other possible new forms of prophylaxis are suggested by studies⁵⁷ on the prevention of the passive sensitization of baboons with allergic human serum by the systemic administration of myeloma IgE, where a refractory state persisting for about a week has been induced by the intravenous injection of about 10 mg protein/kg body weight. The ultimate aim of this approach would be the isolation of a low molecular weight peptide fragment of the myeloma IgE, which retained tissue-binding activity and which could be made available in large amounts by application of modern methods of polypeptide synthesis.

Another advantage to the clinical allergist resulting from the discovery of the myeloma proteins has been the development of methods of measuring IgE concentration which do not rely on tissue or leucocyte sensitization. For example, radioimmunosorbent assay procedures involving the use of anti-IgE²⁴ or specific allergen⁵⁸ coupled chemically to dextran (or some other suitable insoluble carrier such as a cellulose carbonate derivative)⁵⁹ have been used to determine the total, or antibody, IgE levels in the sera of patients, and the use of radiolabelled anti-antibody⁵⁹ has rendered the single radial diffusion test sufficiently sensitive to detect the relatively low levels of IgE in allergic sera by autoradiography.

It is important to recognize, however, that γ E antibodies need not necessarily be deleterious, as suggested, for example, by studies⁶⁰ of the immune reactions occurring in the mucous membranes of the rat during the expulsion of an infecting nematode (*Nippostrongylus brasiliensis*). There is evidence of an association between a sharp rise in intestinal mast cells, an alteration of mucosal permeability (effected by vasoactive amines released from the mast cells) and the expulsion of the parasites. It is tempting to conclude, therefore, in this situation that γ E antibodies are fulfilling a protective role (the so called "self-cure" mechanism⁶¹); and it may be significant that one of the few conditions (other than a hypersensitivity of the asthma-hay fever type, and atopic eczema) where very high serum IgE levels have been recorded is in human (as well as animal) parasitic infections.

- ⁶ Stanworth, D. R., and Kuhns, W. J., quoted in Stanworth, D. R., *Adv. Immunol.*, **3**, 181 (1963).
- ⁷ Hemmings, W. A., and Brambell, F. W. R., *Brit. Med. Bull.*, **17**, 96 (1961).
- ⁸ Tiselius, A., and Kabat, E. A., *J. Exp. Med.*, **69**, 119 (1939).
- ⁹ Brattsten, L., Coldahl, H., and Laurell, A. H. F., *Acta Allergol.*, **8**, 339 (1955).
- ¹⁰ Stanworth, D. R., *Immunology*, **2**, 384 (1959).
- ¹¹ Franklin, E. C., and Stanworth, D. R., *J. Exp. Med.*, **114**, 521 (1961).
- ¹² Heremans, J. F., Heremans, M. T., and Schultze, H. E., *Clin. Chim. Acta*, **4**, 96 (1959).
- ¹³ Stanworth, D. R., *Intern. Arch. Allergy*, **28**, 71 (1965).
- ¹⁴ Vaerman, J. P., Epstein, W., Fudenberg, H., and Ishizaka, K., *Nature*, **203**, 1046 (1964).
- ¹⁵ Fireman, P., Vannier, W. E., and Goodman, H. C., *J. Exp. Med.*, **117**, 203 (1963).
- ¹⁶ Ishizaka, K., Ishizaka, T., and Hornbrook, M., *J. Allergy*, **34**, 395 (1963).
- ¹⁷ Ishizaka, K., and Ishizaka, T., *J. Allergy*, **37**, 169 (1966).
- ¹⁸ Stanworth, D. R., *Adv. Immunol.*, **3**, 181 (1963).
- ¹⁹ Rowe, D. S., and Fahey, J. L., *J. Exp. Med.*, **121**, 171 (1965).
- ²⁰ Terr, A. I., and Bentz, J. D., *J. Allergy*, **35**, 206 (1964).
- ²¹ Andersen, B. R., and Vannier, W. E., *J. Exp. Med.*, **203**, 117 (1963).
- ²² Ishizaka, K., and Ishizaka, T., *J. Allergy*, **42**, 330 (1968).
- ²³ Ishizaka, K., Ishizaka, T., and Hornbrook, M. M., *J. Immunol.*, **97**, 35 (1966).
- ²⁴ Johansson, S. G. O., Bennich, H., and Wide, L., *Immunology*, **14**, 265 (1968).
- ²⁵ Johansson, S. G. O., and Bennich, H., *Immunology*, **13**, 381 (1967).
- ²⁶ Bennich, H., Ishizaka, K., Ishizaka, T., and Johansson, S. G. O., *J. Immunol.*, **102**, 826 (1969).
- ²⁷ Stanworth, D. R., Humphrey, J. H., Bennich, H., and Johansson, S. G. O., *Lancet*, **ii**, 330 (1967).
- ²⁸ Bennich, H., Ishizaka, K., Johansson, S. G. O., Rowe, D. S., Stanworth, D. R., and Terry, W. D., *Bull. World Health Org.*, **38**, 151 (1968).
- ²⁹ Ogawa, M., Kochwa, S., Smith, C., Ishizaka, K., and McIntyre, O. R., *New Engl. J. Med.*, **281**, 1217 (1969).
- ³⁰ Stanworth, D. R., Humphrey, J. H., Bennich, H., and Johansson, S. G. O., *Lancet*, **ii**, 17 (1968).
- ³¹ Stanworth, D. R., Housley, J., Bennich, H., and Johansson, S. G. O. (in preparation).
- ³² Stanworth, D. R., Housley, J., Bennich, H., and Johansson, S. G. O., *Immunochemistry*, **7**, 321 (1970).
- ³³ Sheard, P., Killingback, P. G., and Blair, A. M. J. N., *Nature*, **216**, 283 (1967).
- ³⁴ Van Arsdell, P. P., and Sells, C. J., *Science*, **141**, 1190 (1963).
- ³⁵ Goodfriend, L., and Luhovy, J. I., *Intern. Arch. Allergy*, **33**, 171 (1968).
- ³⁶ Shore, P. A., Burkhalter, A., and Cohn, U. H., *J. Pharmacol. Exp. Ther.*, **127**, 182 (1959).
- ³⁷ Schild, H. O., in *Biochemistry of the Acute Allergic Reactions* (edit. by Austen, K. F., and Becker, E. L.), 99 (CIOMS Symp., 1968).
- ³⁸ Lichenstein, L. M., in *Biochemistry of the Acute Allergic Reactions* (edit. by Austen, K. F., and Becker, E. L.), 153 (CIOMS Symp., 1968).
- ³⁹ Stanworth, D. R., and Henney, C. S., *Immunology*, **12**, 1267 (1967).
- ⁴⁰ Hastie, R., *Clin. Exp. Immunol.* (in the press).
- ⁴¹ Stanworth, D. E., *Immediate Hypersensitivity* (monograph, in preparation).
- ⁴² Henney, C. S., and Stanworth, D. R., *Nature*, **210**, 1071 (1966).
- ⁴³ Stanworth, D. R., *Clin. Exp. Immunol.*, **6**, 1 (1970).
- ⁴⁴ Levine, B. B., *J. Immunol.*, **94**, 111, 121 (1965).
- ⁴⁵ De Weck, A. L., and Schneider, C. H., in *Bayer Symp. on Problems in Allergy*, **1** (1969).
- ⁴⁶ Ishizaka, K., and Ishizaka, T., *J. Immunol.*, **100**, 554 (1968).
- ⁴⁷ Forsgren, A., and Sjöquist, J., *J. Immunol.*, **97**, 822 (1966).
- ⁴⁸ Stanworth, D. R., Matthews, N., and Sjöquist, J. (in preparation).
- ⁴⁹ Matthews, N., and Stanworth, D. R., *Proc. Eighteenth Bruges Symp. Protides of Biological Fluids* (in the press).
- ⁵⁰ Fernö, O., Högborg, B., and Uvnäs, B., *Acta Pharmacol.*, **17**, 18 (1960).
- ⁵¹ Becker, E. L., and Austen, K. F., *J. Exp. Med.*, **124**, 379 (1966).
- ⁵² Lichenstein, L. M., *Proc. Eighth Symp. Collegium Intern. Allergologicum, Switzerland* (1970).
- ⁵³ Catt, K. J., *Lancet*, **i**, 763 (1970).
- ⁵⁴ Jaques, R., *Intern. Arch. Allergy*, **28**, 221 (1965).
- ⁵⁵ Ishizaka, K., and Ishizaka, T., *Clin. Exp. Immunol.*, **6**, 25 (1970).
- ⁵⁶ Patterson, R., *Prog. Allergy*, **13**, 332 (1969).
- ⁵⁷ Stanworth, D. R., *Clin. Allergy*, **1** (in the press).
- ⁵⁸ Vide, L., Bennich, H., and Johansson, S. G. O., *Lancet*, **ii**, 1105 (1967).
- ⁵⁹ Rowe, D. S., *Bull. World. Health Org.*, **40**, 613 (1969).
- ⁶⁰ Miller, H. R. P., and Jarrett, W. F. H. (in the press).
- ⁶¹ Stewart, D. F., *Austral. J. Agric. Res.*, **4**, 100 (1953).
- ⁶² Bennich, H., and Johansson, S. G. O., *Vox Sang.*, **19**, 1 (1970).

¹ Frausnitz, C., and Köstner, H., *Zentr. Bakteriell., Parasiteuk.*, Abt. I, **86**, 160 (1921).

² Coca, A. F., and Grove, E. F., *J. Immunol.*, **10**, 445 (1925).

³ Kuhns, W. J., *Proc. Soc. Exp. Biol. Med.*, **108**, 63 (1961).

⁴ Augustin, R., in *Handbook of Experimental Immunology* (edit. by Weir, D. M.), 1076 (Blackwell, Oxford, 1967).

⁵ Stanworth, D. R., and Kuhns, W. J., *Immunology*, **8**, 323 (1965).

Cyclic Expression of Blood Group Determinants in Murine Cells and their Relationship to Growth Control

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A model for antigenic variation is proposed on the basis of changes in the expression of blood group determinants during the cell cycle.

THERE is a correlation between the growth and surface properties of cells. The division of normal cells in culture is arrested at a critical density¹⁻³. This "density-dependent inhibition"⁴ of growth is less pronounced for tumours⁵ and parallels their degree of malignancy^{6,7}. Alteration of the surface membrane of neoplastic cells has been suggested as a mechanism for defective control of growth^{1,8}. Consistent with such a proposal is the finding that virally transformed fibroblasts possess surface receptors for plant agglutinins^{9,10} which normal fibroblasts express only during mitosis¹¹.

There are, however, several examples of change in the surface properties of tumours during cell division—an increase in electrophoretic mobility of human osteosarcoma cells¹², a decrease of surface immunoglobulin of human lymphomas¹³, a decrease of H-2 alloantigens in the murine cell lines YCAB¹⁴, JLS-V9¹⁵, and the mastocytoma P815Y¹⁶, and an increase of blood group H in HeLa cells during mitosis¹⁷. Such changes may reflect a common surface property of dividing cells.

The expression of blood group A on primary amnion cells in culture also varies with the growth conditions^{17,18}. Franks and Dawson demonstrated that the number of A positive cells in cultures of the rabbit cell line, RK13, was higher during rapid division¹⁹. It was shown, by clonal selection, that antigen negative cells gave rise to both positive and negative progeny. I describe here the expression of blood group determinants B and H in synchronized cultures of the mouse mastocytoma P815Y and phytohaemagglutinin (PHA)-treated mouse lymphocytes, and propose a model for antigenic variation during cell division.

The mouse mastocytoma P815 strain Y was maintained in suspension culture in Fischer's medium supplemented with 10% horse serum. Cell synchrony was achieved by the double thymidine block procedure²⁰, as follows: an exponential culture (2×10^5 cells/ml.) was grown for 15 h with 2×10^{-3} M thymidine, 6.5 to 8 h in its absence and a further 8 h with 2×10^{-3} M thymidine. The cells were reincubated in fresh medium at a cell density of $2-3 \times 10^5$ /ml. The degree of synchrony was monitored by serial cell counts and measurement of ³H-thymidine incorporation into DNA. Cells had a mean generation time of 15 h.

Mouse mesenteric node lymphocytes were cultured with PHA (Wellcome Reagents Ltd, reagent grade, reconstituted

to 5 ml. with distilled water) in Eagle's minimum essential medium (MEM) supplemented with 10% foetal calf serum. Control cultures were treated with PHA for 3 h before collection.

Cells were stained for blood groups B and H by indirect immunofluorescence. Cell suspensions (2×10^6 ; 200 μ l.) were incubated with an equal volume of antiserum at the appropriate dilution (1/100 to 1/200) for 30 min at 3° C, washed three times with cold MEM, and incubated for 30 min at 3° C with 50 μ l. of fluorescein-conjugated rabbit anti-human gamma-globulin (1/20 dilution, previously absorbed with an equal volume of mastocytoma cells). Cells were washed a further four times with cold MEM and examined with a Leitz Ortholux microscope under ultraviolet light. Controls for serological specificity were antisera absorbed with an equal volume of A-positive or B-positive human erythrocytes, or anti-A antiserum. Anti-B antiserum (Lot No. 70-52103) was a gift from Ortho Pharmaceuticals and anti-H antiserum ('Baxter' serum, activity for O and A human erythrocytes) was a gift from Dr K. L. G. Goldsmith, MRC Blood Group Reference Laboratory. For the determination of mitotic index, cells were lysed with 1% sodium citrate and the nuclei washed with cold methanol-acetic acid mixture (3:1 v/v). Air-dried smears were stained with May-Grünwald-Giemsa.

In P815Y cells, the expression of blood group determinants B and H was related to growth rate (Fig. 1). Resting cells (late log phase) were negative for B and positive for H. On transfer to fresh medium, an inverse change occurred in antigen expression during the exponential phase of growth. It was decided to study this event during the period of the cell cycle.

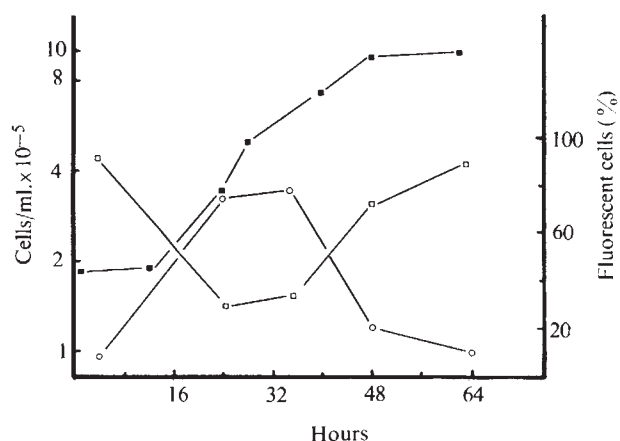
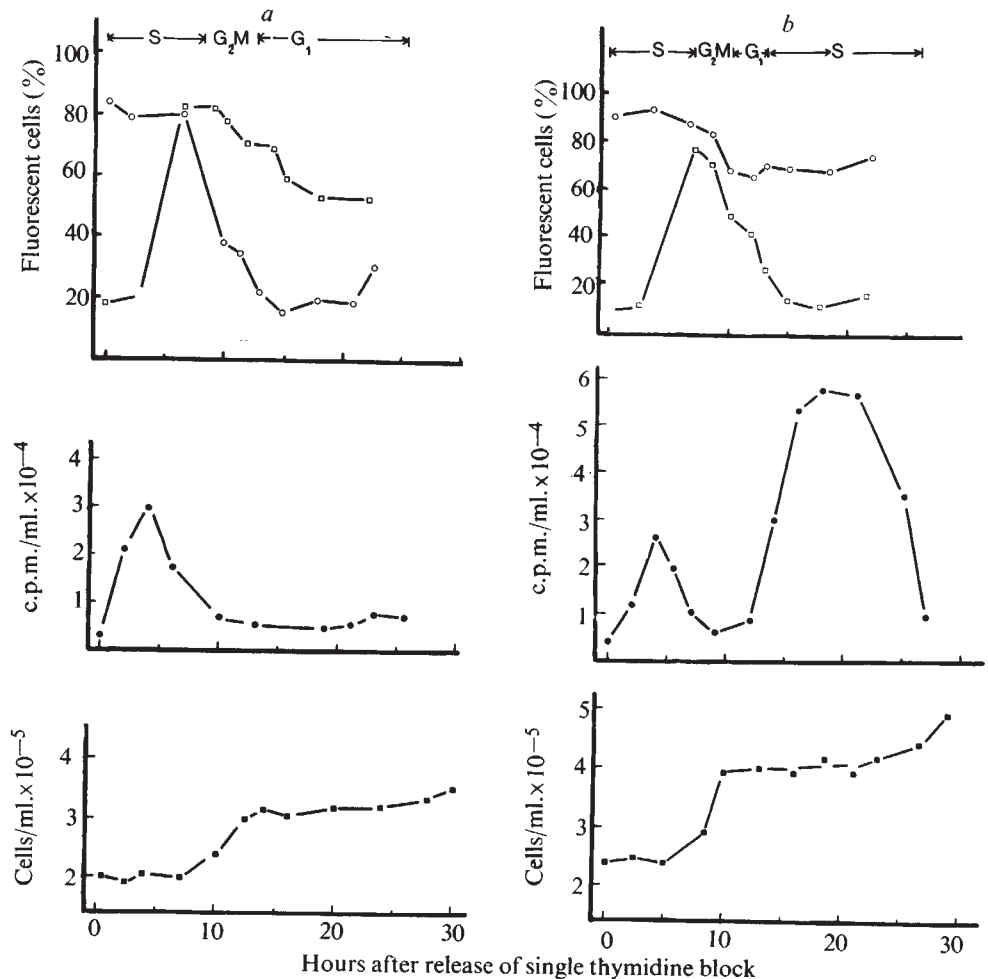


Fig. 1 Expression of blood group determinants B and H in an asynchronous culture of P815Y cells. ■, Cell number; ○, B positive cells; □, H positive cells.

Fig. 2 Expression of blood group determinants B and H in synchronized P815Y cells. Conditions of cell culture were identical apart from the time of the second thymidine addition (7.75 h for *a*; 7 h for *b*). Cell samples (1 ml.) were removed at intervals and incubated with ^3H -thymidine (0.4 $\mu\text{Ci}/\text{ml.}$) for 30 min. Cells were washed with MEM and the DNA was precipitated with 5% trichloroacetic acid. Precipitates were dispersed in 1% 'Triton X-100' and counted in a liquid scintillation spectrophotometer using a toluene phosphor. Samples of synchronized cells were analysed for cell number (■), ^3H -thymidine incorporation (●), blood group B (○), and blood group H (□).



In preliminary experiments, using the double thymidine block procedure, it was possible to obtain synchrony for two cell divisions occasionally (2 in 5). Failure to obtain more than one synchronous division is a common feature of different cell lines and synchrony procedures; the reasons remain obscure²¹. Appearance of a second peak of DNA synthesis was critically dependent on the release period between the first and second thymidine blocks. Also, the degree of synchrony was influenced by cell contact, spinner cultures being more successful than stationary cultures. This is illustrated in Fig. 2 where change in B and H expression was examined in cultures giving one (Fig. 2a) or two (Fig. 2b) synchronous cell divisions. Conditions of cell culture were identical apart from the time of the second thymidine addition; the sample in Fig. 2a was maintained as a stationary culture for the duration of the second thymidine block. In the absence of a second peak of DNA synthesis (Fig. 2a), G_1 cells were B^- and H^+ . However, this is not always a feature of G_1 and the majority of cells in the second culture (Fig. 2b) were B^+ and H^- in this phase. Decay of synchrony and inability to express B was not a consequence of thymidine cytotoxicity. G_1 (B^- and H^+) cells were viable ($>98\%$ by dye exclusion tests) and exhibited asynchronous growth after a lag period of several hours. To decide whether the above changes occurred at mitosis or an early part of G_1 , cells were released from a single thymidine block and cultured in the presence of the mitotic inhibitor, colcemid (Fig. 3). It is seen that H is maximal at mitosis. No quantitative difference was found for B expression in the interval between thymidine removal and complete mitotic arrest; different samples were equally positive for B at all dilutions of antiserum. Therefore expression of B and H at G_1 is related to the ability of cells to undergo further synchronous division. The difference in staining pattern for blood groups B and H in P815Y cells is illustrated in Fig. 4.

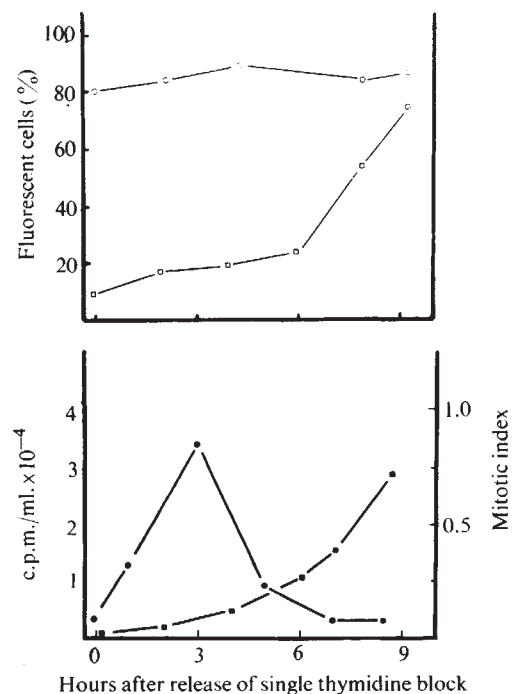


Fig. 3 Expression of blood groups B and H in P815Y cells after release of single thymidine block. An exponentially growing culture (2×10^5 cells/ml.) was exposed to 2×10^{-3} M thymidine for 15 h. Cells were washed and resuspended in fresh medium containing colcemid (0.04 $\mu\text{g}/\text{ml.}$). Samples were removed at intervals and examined for ^3H -thymidine incorporation (●), mitotic index (■), blood group B (○), and blood group H (□).

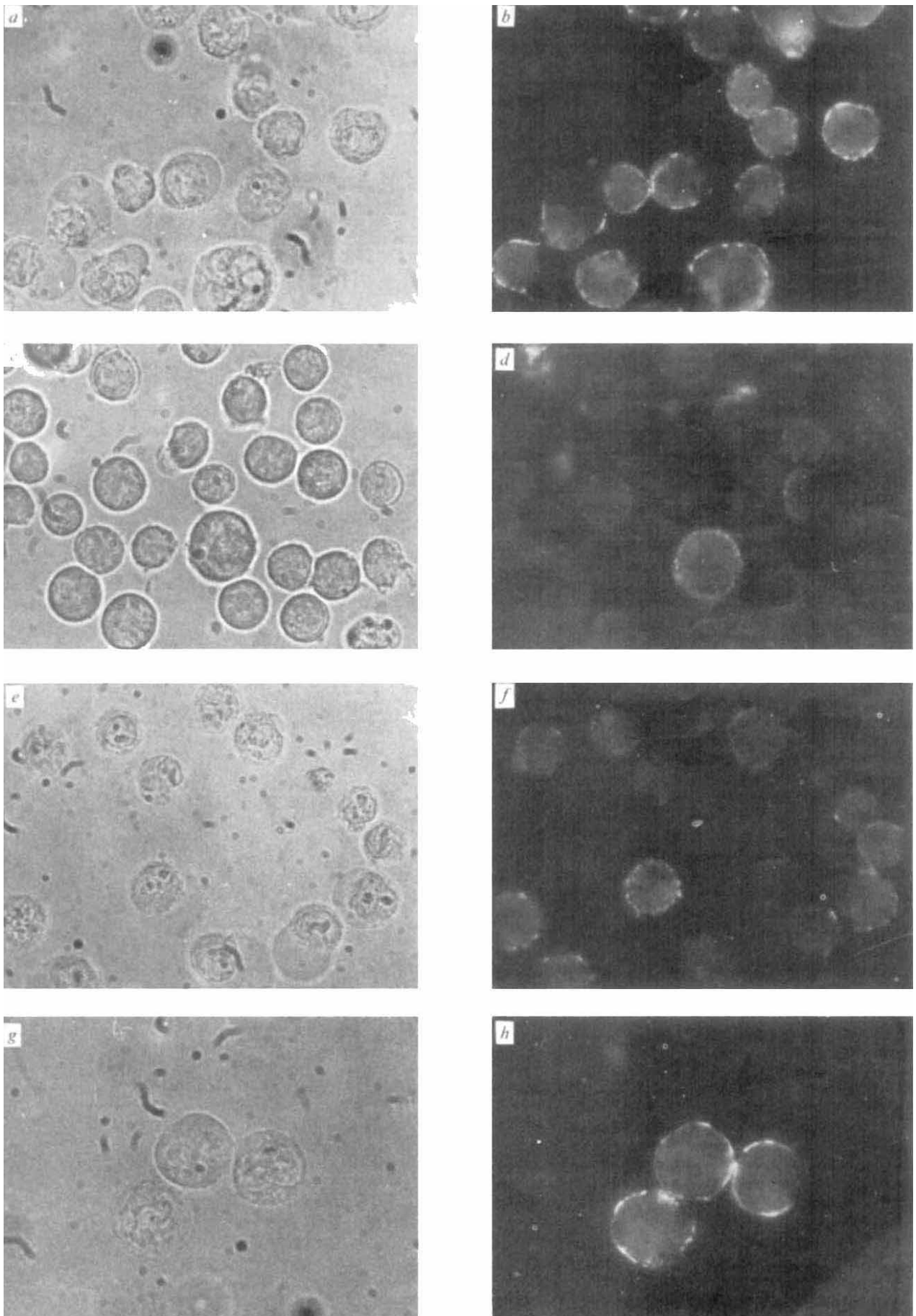


Fig. 4 Immunofluorescent staining of synchronized P815Y cells: *a* and *b*, H positive cells at G_1 ; *c* and *d*, B positive and negative cells at G_1 ; *e* and *f*, H positive and negative cells at S; *g* and *h*, B positive cells at S.

Preliminary experiments suggest that a similar change in B expression occurs in synchronized cultures of the murine leukaemia, L1210. Resting cells are B⁻, thymidine blocked cells are B⁺, and G₁ cells are B⁻ after one synchronous division. I have excluded the possibility that this restricted expression is only a feature of tumours by showing a similar occurrence for normal mouse by lymphocytes.

Lymphocytes represent a homogeneous population of resting, G₀, cells which divide only on contact with specific antigen or mitogenic agents such as the plant agglutinin, PHA. Also mouse lymphocytes are largely B⁻ (7–10% B⁺ cells in mesenteric lymph nodes). Table 1 shows that B⁺ cells were present in lymphocyte cultures after incubation with PHA. The number of positive cells was related to the PHA concentration, being maximal at or near the optimum mitogenic dose (Table 2). At earlier times (48 h, Table 1) the number of B⁺ cells exceeded that of ³H-thymidine-labelled cells. This finding suggests that expression of B is an early event of blast cell transformation. Appreciable agglutination of lymphocytes occurred after incubation with PHA and estimation of cell numbers was approximate. However, each cell with a typical lymphoblastoid appearance under phase contrast microscopy was also fluorescent. Appearance of B on PHA-treated lymphocytes is not strain specific; similar results were obtained with the strains Balb/C, CBA, C3H and DBA/2 all of which showed >20% B⁺ cells at 48 h of culture. The occurrence of this surface change may prove useful as an additional index of lymphocyte transformation.

It was not possible to examine the spatial relationship of B to H-2 alloantigens on the cell surface by blocking procedures with specific antisera. The pretreatment of B⁻, P815Y cells with anti-H-2^d (C3H anti-DBA/2) antiserum gave a positive result on subsequent immunofluorescent staining for blood group B. This may be due to cross-reaction of mouse immunoglobulin with the B determinant.

The chemical basis of serological specificity is established for the ABO system²²; thus blood group antisera provide useful reagents for detecting the presence of specific sugar residues at the cell surface. The fortuitous cross-reaction of murine antigens with B and H determinants does not imply identity with the parent blood groups. It can be concluded only that a glycolipid or glycoprotein moiety with terminal α -galactosyl 1→3 galactose (B specificity) or α -fucosyl 1→2 galactose (H specificity) residues is present on the cell surface. It is a matter of conjecture whether appearance of B requires *de novo* synthesis or exposure of a pre-existing, cryptic antigen¹⁰. The restricted expression of B may not be a general feature of murine cells. Thus the thymus has a high mitotic rate, yet only 8–10% of thymocytes are B⁺ by indirect immunofluorescence.

Table 1 Appearance of Blood Group B Positive Cells in Mouse Lymphocyte (Balb/c Strain) Cultures after Treatment with PHA (1/100 Dilution)

Time of culture (h)	Control cultures		PHA-treated cultures	
	% B ⁺ cells	% ³ H-thymidine-labelled cells	% B ⁺ cells	% ³ H-thymidine-labelled cells
0	7–10	0	7–10	0
24	3	<1	8–10	<1
48	<2	<1	20–25	7–10
72	<2	<1	20–25	24–28

* Determined by autoradiography after exposure of cells to ³H-thymidine (1 μ Ci/ml.) for 3 h.

Table 2 Effect of PHA Concentration on the Mitogenic Response and Expression of Blood Group B in Lymphocyte (Balb/c Strain) Cultures at 72 h

PHA concentration	% B ⁺ cells	³ H-thymidine incorporation* (c.p.m./ml. $\times 10^{-3}$)
0	<1	0.65
1/500	5–10	6.0
1/200	20–25	19.7
1/100	20–25	35
1/50	<5	3

* Cells (2×10^6 /ml.) were incubated with ³H-thymidine (1 μ Ci/ml.) for 3 h and the trichloroacetic acid-insoluble radioactivity determined by scintillation counting.

In P815Y cells, there is an inverse relationship between expression of H-2 alloantigens and blood group H on the one hand, and blood group B on the other. H-2 has been shown to be minimal during the intermitotic period¹⁶, in agreement with a reported variation of H-2 and viral antigens in other murine cell lines^{14,15}. Variation in surface antigens during cell division has been accounted for by a limited period of gene transcription and/or decrease in rate of synthesis^{14–16}. However, the appearance of a new determinant, B, suggests that decrease of H-2 and H might be due to a conformational change at the cell surface. A similar proposal has been made to account for change in surface charge and electrophoretic mobility during cell division²³.

The expression of H is maximal in synchronized HeLa cells at mitosis, but for surface antigens in other cell lines the peak occurs in G₁^{14,15}. Cikes and Friberg have tried to reconcile this difference by suggesting that the appearance of H was an early G₁ event¹⁵. Yet the present studies show that, according to degree of synchrony (Fig. 2), cells can proceed directly at mitosis from an H⁺ state to an H⁻G₁ state. Thus the antigenic

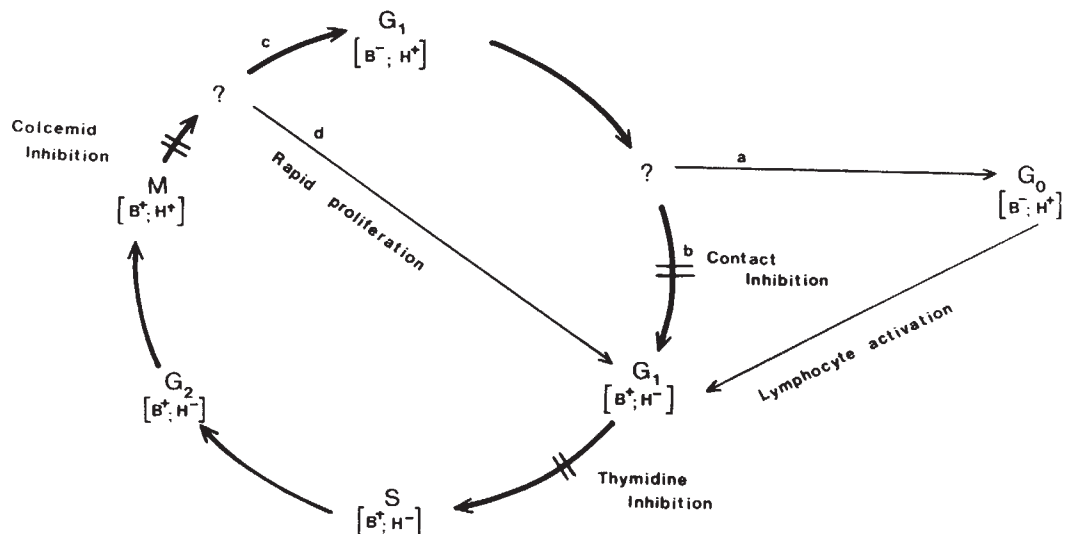


Fig. 5 Proposed model for antigenic variation during the cell cycle. B⁺ is α -galactosyl 1→3 galactose; H⁺ is α -fucosyl 1→2 galactose.

properties of G_1 cells may reflect a potential for further division.

Model for Antigenic Variation

I wish to propose a model for antigenic variation during the division of P815Y cells and the PHA-induced transformation of mouse lymphocytes (Fig. 5). The evidence in support of the scheme is as follows. (1) Resting cells (G_1 or G_0) are B^- and H^+ and give rise to B^+ and H^- cells during exponential growth (Fig. 1). (2) At mitosis, cells can proceed to a G_1 state which is B^- and H^+ (Fig. 2a) or B^+ and H^- (Fig. 2b). (3) G_1 cells (B^+ and H^- , Fig. 2b) undergo synchronous division while G_1 cells (B^- and H^+ , Fig. 2a) are restricted to asynchronous growth. (4) Blood group B determinant is expressed on lymphocytes after treatment with PHA, and its appearance precedes DNA synthesis (Table 1).

Accordingly, the ability to express the α -galactosyl 1 $\rightarrow$ 3 galactose moiety (B determinant) is a prerequisite for cell division. It is not claimed that changes in B and H expression initiate cell division, but rather that they provide an index of a cell with commitment to mitosis. Two branch points exist in the cycle (a + b; c + d) which may serve as sites of growth control. Escape from growth inhibition is associated with the permanent expression of B at the cell surface (Fig. 2b). In limiting conditions of growth, there is an increase in the number of B^- cells (Fig. 1). It is suggested that cells, at mitosis, can be diverted from a path of rapid proliferation (d) to one of growth inhibition (c) with arrest in a G_1 (or G_0) B^- state.

It is known that variation in division time of cells, *in vivo* and *in vitro*, can be accounted for by differences in the G_1 period, the sum of S + G_2 + M being constant^{24,25}. Also, G_1 is absent for some cells during rapid proliferation²⁶. The possibility of two pathways, c and d, for cell division would provide a basis for this variation in the G_1 interval.

Furthermore, it is reasonable to expect that differences in

surface properties of rapidly dividing (tumour, embryonic ?) cells and other cells would be most apparent in G_1 and would be minimal in limiting conditions of growth.

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- ¹ Abercrombie, M., and Ambrose, E. J., *Cancer Res.*, **22**, 525 (1962).
- ² Eagle, H., *Science*, **148**, 42 (1965).
- ³ Levine, L., Becker, Y., Boone, C., and Eagle, H., *Proc. US Nat. Acad. Sci.*, **53**, 350 (1965).
- ⁴ Stoker, M. G. P., and Rubin, H., *Nature*, **215**, 171 (1967).
- ⁵ Todaro, G. J., and Green, H. J., *J. Cell Biol.*, **17**, 299 (1963).
- ⁶ Pollack, R. E., Green, H., and Todaro, G. J., *Proc. US Nat. Acad. Sci.*, **60**, 126 (1968).
- ⁷ Aaronson, S. A., and Todaro, G. J., *Science*, **162**, 1024 (1968).
- ⁸ Wallach, D. F. H., *Proc. US Nat. Acad. Sci.*, **61**, 868 (1968).
- ⁹ Burger, M. M., and Goldberg, A. R., *Proc. US Nat. Acad. Sci.*, **57**, 359 (1967).
- ¹⁰ Burger, M. M., *Proc. US Nat. Acad. Sci.*, **62**, 994 (1969).
- ¹¹ Fox, T. O., Sheppard, J. R., and Burger, M. M., *Proc. US Nat. Acad. Sci.*, **68**, 244 (1971).
- ¹² Mayhew, E., *J. Gen. Physiol.*, **49**, 717 (1966).
- ¹³ Buell, D. N., and Fahey, J. L., *Science*, **164**, 1524 (1969).
- ¹⁴ Cikes, M., *J. Nat. Cancer Inst.*, **45**, 979 (1970).
- ¹⁵ Cikes, M., and Friberg, S., *Proc. US Nat. Acad. Sci.*, **68**, 566 (1971).
- ¹⁶ Pasternak, C. A., Warmsley, A. M. H., and Thomas, D. B., *J. Cell Biol.* (in the press).
- ¹⁷ Kuhns, W. J., and Bramson, S., *Nature*, **219**, 938 (1968).
- ¹⁸ Dawson, A., and Franks, D., *Exp. Cell Res.*, **47**, 377 (1967).
- ¹⁹ Franks, D., and Dawson, A., *Exp. Cell Res.*, **42**, 543 (1966).
- ²⁰ Galvazi, G., and Bootsma, D., *Exp. Cell Res.*, **41**, 438 (1966).
- ²¹ Peterson, D. F., and Anderson, E. C., *Nature*, **203**, 642 (1964).
- ²² Watkins, W. M., in *Glycoproteins*, BBA Library (edit. by Gottschalk, A.), **5**, 462 (Elsevier, Amsterdam, 1966).
- ²³ Kraemer, P. M., *J. Cell Biol.*, **33**, 197 (1967).
- ²⁴ Cameron, I. L., and Greulich, R. C., *J. Cell Biol.*, **18**, 31 (1963).
- ²⁵ Prescott, D. M., *Cancer Res.*, **28**, 1815 (1968).
- ²⁶ Robbins, E., and Schraff, M. D., *J. Cell Biol.*, **34**, 684 (1967).

Cytostatic Antibody and SV40 Tumour Immunity in Hamsters

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Ambrose *et al.* propose a likely scheme for the course of tumour development, as a failure of the host immune system.

THERE is growing support for the view that cancer involves a failure of the immune system of the host¹, and we have set out to investigate the mechanism of this failure in newborn hamsters injected with SV40 virus. For our purposes, this system has the advantage that autochthonous tumours occur reproducibly, that tumourigenesis can be prevented by immunization (which shows that the immune system of the host is functional before

the occurrence of tumours) and that all tumours have the same transplantation antigens (TATAS).

Tumours occur reproducibly after a latent period of 90 to 300 days, during which the appearance of tumours can be prevented either by a second SV40 injection or immunization with irradiated SV40 tumour cells². In previous studies, we attempted to delineate the roles of cell-mediated immunity and humoral immunity in this response. The occurrence of cell-mediated immunity is demonstrated by the way in which lymphocytes from immune animals will protect non-immune animals against challenge by tumour cells³, but the role of humoral immunity is more obscure. Circulating cytotoxic antibodies have been reported in patients recovering from Burkitt's tumour⁴, and there is some evidence that what are called blocking antibodies

will enhance the growth of tumours, either by reacting with target cells⁶ or with immune lymphocytes⁷.

To distinguish between cell-mediated and humoral immunity *in vivo*, we have measured quantitatively the growth of tumour cells in small plastic chambers which are implanted in the peritoneal cavity and which are permeable to antibodies, not to cells^{8,9}. After several days, the chambers are removed and the viable cells are counted. In non-immune animals, the number of cells increases, but in animals which are immune, growth is retarded or even prevented. So long as the humoral factors have not been isolated in pure form, we prefer to describe humoral factors defined by this method as cytostatic and not cytotoxic.

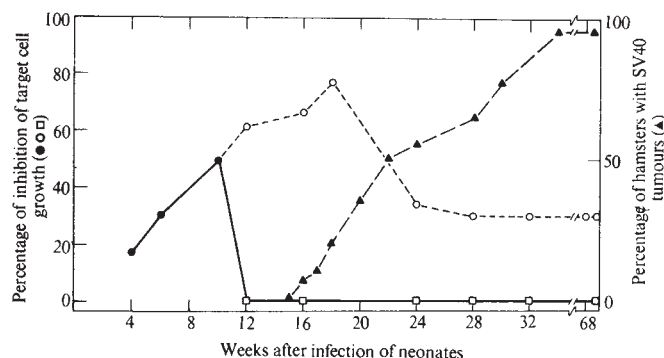


Fig. 1 Detection of cytostasis of SV40 tumour cells in diffusion chambers implanted in hamsters at intervals following neonatal infection with SV40 virus. ●, Cytostatic antibody in all animals; ○ tumour free animals; □, pre-tumour animals; ▲, palpable tumours.

In our experiments, we use animals of the LVG/LAK strain of Syrian golden hamsters. We produce autochthonous tumours by injecting newborn hamsters in the right subscapular space with 0.2 ml. doses of a suspension of SV40 virus (Fig. 1). Palpable tumours begin to appear at the site of injection after about 90 days, and 95% of the hamsters develop tumours after 200–300 days. To immunize adult hamsters so as to interrupt oncogenesis, three 0.2 ml. doses of the same virus suspension are injected at weekly intervals.

Antibody in Latent Period

One series of experiments was designed as a test for circulating antibody in the latent period before the appearance of tumours. After weaning at 21 days, we tested each week a group of ten animals (mixed sex groups) for circulating antibody, using as controls animals injected at birth with green monkey kidney passage control fluid medium containing no SV40 virus. Implanted chambers containing SV40 tumour cells were removed after 5 days and the animals were palpated for tumours at weekly intervals.

In the first 10 weeks of life, there is a surprising growth of circulating antibody in all ten infected animals in each infected group. Thereafter, there is a precipitous decline of circulating antibody in those animals which later develop tumours, with the loss of antibody always preceding the appearance of the tumour. Animals which remain free from tumours after 500 days still have measurable amounts of circulating antibody.

The transient occurrence of circulating antibody implies that the corresponding antigen is functional, which in turn implies that latency cannot be accounted for by dormancy of the virus. Instead, it seems as if transformed cells are produced and that they remain quiescent during latency, and we have been able to demonstrate that such cells do indeed exist.

To this end, we have been able to use the intracellular antigen called the T antigen which is produced when cells are transformed by SV40 virus and which may be detected with fluorescent antibodies^{10,11}. The antigen is also produced during

lytic infections¹² but is not always associated with oncogenicity, since cells transformed with SV40 virus but lacking T antigen have been found. Even so, the T antigen is a useful marker for cells which have been infected (but possibly not transformed) with SV40.

The inhibition relative to the control target cells is shown in Fig. 1.

Hamsters injected subcutaneously at birth with SV40 were killed in groups of ten, first at 8 h and then at 24 h intervals after injection. The tissue in the rejection area (5–8 mm in diameter) was sectioned and examined for T antigen using fluorescein conjugated antibody (Flow Laboratories). Within 8 h, approximately 10% of the cells examined showed strong diffuse immunofluorescence in the cytoplasm and a bright halo around the nuclear membrane. Cells from control hamsters injected with virus-free culture fluid were uniformly negative. In infected animals, more than 90% of the cells in the connective tissue region showed cytoplasmic fluorescence by 48 h but on the fourth day, few cells showed specific staining in either the nucleus or the cytoplasm, suggesting abortive infection. There was no evidence of cell degeneration. Intra-nuclear fluorescence was seen in a few pockets of six to eight cells after 7 days, but these numbered less than 5% of the cells in the injection area. During the next 17–20 days, cytoplasmic staining disappeared in the T antigen-positive cells and nuclear fluorescence weakened. A new type of large cell never seen in control sections was, however, observed to emerge among the positive cells. These cells, which formed foci and increased in number after 30 days are thought to give rise to tumours.

This work demonstrates that infected and presumably transformed cells persist in fairly large numbers during the latent period and produce the antigen or antigens giving rise to circulating antibody. As competence to produce humoral antibody appears before that for cellular immunity¹⁴, circulating antibody may be produced initially and may inhibit a cellular response afferently or efferently.

To define the role of humoral factors more closely, we ask whether serum containing circulating antibody can inhibit SV40 tumour cell proliferation when administered passively in implantation chambers.

Table 1 Detection of Cytostatic Antibody against SV40 Target Cells following Passive Administration of Serum to Normal Hamsters

Serum donor	Average No. viable cells/chamber after 5 days' incubation*	% cytostasis compared with normal serum control
Normal control	618,000	
Hyperimmunized to SV40 tumour	327,000	47
SV40 tumour bearer	596,333	5

* Chambers seeded with 50,000 viable SV40 tumour cells.

SV40 target cells were incubated for 1 h *in vitro* in sera from normal immunized hamsters and from animals with tumours and then inoculated into diffusion chambers placed in weanling hamsters. Ten experimental and ten control animals were given 0.2 ml. of immune or control serum each day for 4 days. On the fifth day, the chambers were removed and the viable cells counted (Table 1). Normal serum gave a typical cell increase over 5 days, whereas animals receiving serum from SV40-tumour-immune hamsters showed cytostasis comparable with that observed in chambers implanted in immunized donors. In contrast, passive administration of serum from tumour bearers produced only slight inhibition of tumour cell growth. This confirms that a humoral factor plays a part in cytostasis but is absent or markedly decreased in tumour bearers. So far, the correlation between circulating antibody and the absence of a palpable tumour is complete, but the role of the circulating antibody remains obscure.

Course of Antibody Production

Does circulating antibody invariably follow immunization? To test this, the time course of appearance of circulating antibody was followed in adult hamsters after a single intraperitoneal injection of either 10^7 irradiated (5,000 r.) SV40 tumour cells or SV40 virus (Fig. 2). Circulating antibody was detected after five days, increasing to 65% inhibition and falling to a low level, followed by a slow rise in animals immunized with tumour cells. Baboon anti-hamster IgG serum labelled with fluorescein was used to show that cells recovered from chambers at 24 days were coated with IgG while those recovered at 8 days were not. Unfortunately, anti-hamster IgM serum was not available to determine whether the inhibition observed between 4 and 12 days was due to IgM, but it seems reasonable to make this assumption. The SV40 virus response apparently is due only to IgG. Animals immunized to both irradiated tumour cells* and virus were resistant to SV40 tumour cell challenge.

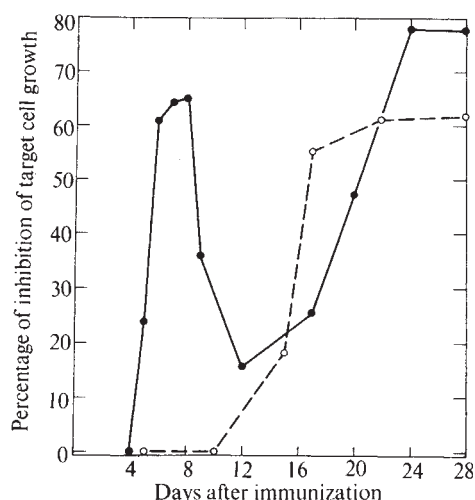


Fig. 2 Time course of appearance of cytostatic antibody against SV40 tumour cells in diffusion chambers in hamsters immunized with either irradiated SV40-transformed tumour cells grown in tissue culture or SV40 virus. ●, SV40 tumour cells (5,000 r.); ○, SV40.

The rapidity of the immune response observed with irradiated cell immunization raises the question of whether a transient circulating antibody response also occurs when nonimmunized animals are challenged with tumour-producing doses of SV40-transformed cells.

SV40 tumour cells (5×10^4 viable cells) were injected subcutaneously in the subscapular region of weanling hamsters. Before this and at 5 day intervals afterwards, diffusion chambers containing SV40 tumour cells were implanted into the peritoneal cavity; and 5 days later the number of tumour target cells was counted (Fig. 3). Circulating antibody was detected by 5 days after injection and persisted for approximately 10 days in all animals. No circulating antibody could be detected in any hamster by 15 days, at which time 25% of the challenged animals had very small palpable masses at the site of tumour cell injection. All animals developed tumours by 20 days.

In this system, therefore, circulating antibody invariably precedes tumour appearance, slows tumour cell growth, and is always present in animals immune to tumour challenge. Does it reappear when tumours are removed?

* We observed that splenectomy performed before multiple immunization of hamsters with irradiated SV40 tumour cells had no effect on circulating antibody formation, whereas splenectomy following immunization completely eliminated pre-existing levels of antibody. Both splenectomized animal groups resisted subsequent SV40 tumour challenge.

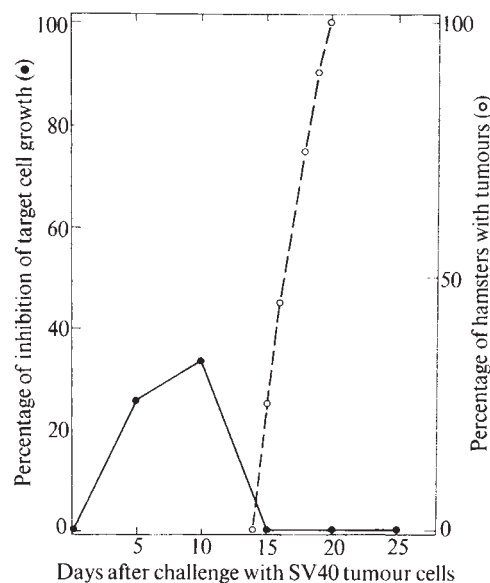


Fig. 3 Transient appearance of cytostatic antibody to SV40 tumour cells in diffusion chambers after implantation of live unirradiated SV40 tumour cells. ●, Cytostatic antibody; ○, palpable tumours.

Postsurgical Immune Response

Four weeks after injection of SV40 tumour cells into adult hamsters, a surgical cure was attempted. All animals had been monitored for circulating antibody, and none was found. Considerable attention to detail is required to achieve complete tumour removal. The cells used to produce tumours were injected subcutaneously in 0.1 ml. (5×10^4 cells) in a shaved area near the dorsal midline. Care was taken to minimize cell leakage or penetration of injected cells into adjacent tissues. When the fibrosarcomas reached 1–2 cm in diameter, the area was again shaved, the skin cleansed, and the tumour with considerable adjacent tissue lifted well away from the trunk and clamped off. The tumour and as much adjacent normal tissue as practical was cut away at the haemostat, and the wound was closed with clips, leaving the skin taut in the area of surgery. With these precautions and with care to avoid tumour cell dispersion *in situ* by vigorous palpation, a 50 to 60% cure rate could be achieved. Animals tumour free for 150 days after surgery were considered cured.

When circulating antibody levels were determined, about half the animals recovered after 10 days, which coincided with high levels of circulating antibody (Fig. 4). Results from all animals were pooled and plotted as a single result for each group, as the variation in cell counts was small for tumour-free animals ($\pm 5\%$).

Basis of Immune System Failure in Cancer

The conclusion that so called tumour-specific transplantation antigens, including those appearing after oncogenic virus infection, may be normal early embryonic antigens re-expressed in the tumour is reviewed in detail elsewhere^{15–18}. The idea is based on four lines of evidence: (1) antisera to tumour surface antigens react with a variety of embryonic cells; (2) irradiated early embryonic cells immunize adult animals against a variety of tumours including transplanted, autochthonous, spontaneous, and viral-induced tumours and elicit humoral and cellular responses against tumour detected *in vitro*; (3) during and after pregnancy circulating antitumour antibodies and lymphocytes are found; and (4) heterologous antisera to a wide variety of chemically induced tumours, after absorption with normal adult donor tissues, react with foetal cells. The para-

doxical problem inherent in the concept of immunologic surveillance is to reconcile why the foetal allograft, tolerated during normal pregnancy, is not rejected. Does a developing cancer survive by a similar mechanism, albeit outside the placental "barrier"?

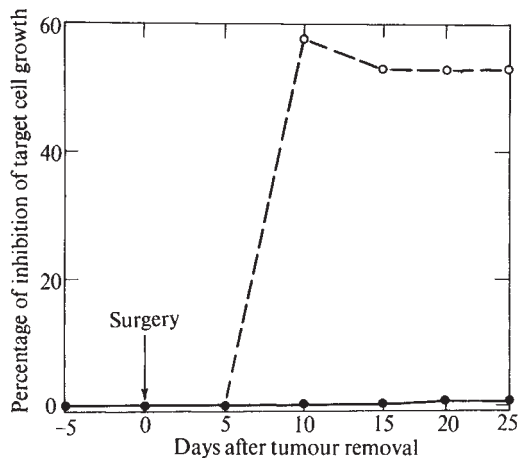


Fig. 4 Appearance of cytostatic antibodies after successful surgical removal of tumours (○) and continued absence of antibodies in surgical failures (●).

A solidly effective immune response to naturally occurring neoplasia may be difficult to achieve in practice, and may actually be effective only for very small groups of cells, as the data presented here suggest. We may divide the time course of tumour induction with SV40 virus into four phases: (1) Initial oncogenicity: a small tumour mass is established following virus infection which exhibits embryonic antigens. The cells may grow unrestricted for a short period (a few hours) before an immune response occurs. (2) Cytostatic immunity: some cells expressing tumour antigens may be destroyed during the initial antibody response; however, tumour cells are present for which the circulating antibodies are cytostatic and not cytotoxic. Numerous studies in our laboratory for many tumour systems in hamsters and mice revealed that *in vivo* humoral responses to tumour cells are cytostatic in nature^{8,9,14,15}. It should be noted that the demonstration of cytotoxic antibodies usually requires a specially selected xenotypic complement to be effective^{19,20} and that cytotoxicity is demonstrated experimentally *in vitro*. The result is a long latent period of very slow growth. During this period immunization with irradiated cells or virus is effective in preventing tumour formation. (3) Transition to local antigen excess: at some critical tumour mass, antigen production is thought to be sufficient to create a condition of local antigen excess, and rapid tumour growth ensues. As antigen excess becomes widespread, circulating antibody to the antigen becomes undetectable. (In preliminary experiments ¹³¹I-labelled, tumour-eluted antibody injected into tumour bearers was rapidly localized in the kidneys as has been shown to occur with soluble antigen-excess complexes.) (4) Uncontrolled growth: once the circulating antibody is outstripped, the tumour mass increases rapidly. After successful surgery, however, the antibody rapidly reappears. Functional lymphocytes are present in tumour-bearing animals capable of destroying tumour cells *in vitro*^{21,22}, but these fail to eliminate the tumour. This failure most probably results from immunological "confusion" perpetuated by circulating antigen or antigen-antibody complexes at the lymphocyte surface. Circulating antibody may thus play a role as a masking factor preventing activation of both cell-mediated immunity and lymphocyte or macrophage function.

This is the simplest description consistent with the observations. Whether other tumours follow a similar time course remains to be determined. This description does not emphasize cell-mediated immunity, largely because it was not measured. However, both adoptive transfer of immunity with immune lymphocytes and colony inhibition studies have demonstrated a cellular defence mechanism against SV40 virus tumours^{3,20,21}.

Implications for Human Cancer

Many human cancers, including carcinoma of the cervix and prostate, have long latent periods during which cytostatic antibodies may appear. When rapid growth occurs, the antibody level should drop, to reappear again after successful treatment²².

If the SV40 model studied here is a valid model for immunologic reactivity to autochthonous tumours in man, then the possibility of detecting cancer in the period of cytostatic antibody control exists.

We have not invoked here a multiplicity of different antibodies including enhancing or special blocking antibodies as distinct from cytotoxic ones. Rather, circulating antibody is thought to both depress tumour growth and prevent immune lymphocytes from being effective. In the latter role, these antibodies may produce enhancement. To evaluate the validity of this view, cytostatic antibodies and tumour surface antigens must be available in a high state of purity.

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- Hellström, K. E., and Hellström, I., *Adv. Cancer Res.*, **12**, 167 (1969).
- Coggin, jun., J. H., Larson, V. M., and Hilleman, M. R., *Proc. Soc. Exp. Biol. Med.*, **124**, 774 (1967).
- Coggin, jun., J. H., Larson, V. M., and Hilleman, M. R., *Proc. Soc. Exp. Biol. Med.*, **124**, 1295 (1967).
- Hellström, I., Hellström, K. E., and Pierce, G. E., *Intern. J. Cancer*, **3**, 467 (1968).
- Herberman, R. B., and Fahey, J. L., *Proc. Soc. Exp. Biol. Med.*, **127**, 938 (1968).
- Hellström, I., and Hellström, K. E., *Intern. J. Cancer*, **4**, 587 (1969).
- Hellström, K. E., and Hellström, I., *Ann. Rev. Microbiol.*, **24**, 373 (1970).
- Coggin, jun., J. H., and Ambrose, K. R., *Proc. Soc. Exp. Biol. Med.*, **130**, 246 (1969).
- Ambrose, K. R., Candler, E. L., and Coggin, jun., J. H., *Proc. Soc. Exp. Biol. Med.*, **132**, 1013 (1969).
- Pope, J. H., and Rowe, W. P., *J. Exp. Med.*, **120**, 121 (1964).
- Rapp, F., Kitahara, T., Butel, J. S., and Melnick, J. L., *Proc. US Nat. Acad. Sci.*, **52**, 1138 (1969).
- Levin, M. J., Oxman, M. N., Diamandopoulos, G. T., Levine, A. S., Henry, P. H., and Enders, J. F., *Proc. US Nat. Acad. Sci.*, **62**, 589 (1969).
- Diamandopoulos, G. T., Tevethia, S. S., Rapp, F., and Enders, J. F., *Virology*, **34**, 331 (1968).
- Hutchins, P., Amos, D. B., and Prioleau, W. H., *Transplantation*, **5**, 68 (1967).
- Coggin, jun., J. H., Ambrose, K. R., Bellomy, B. B., and Anderson, N. G., *J. Immunol.* (in the press).
- Coggin, jun., J. H., Ambrose, K. R., and Anderson, N. G., *Proc. First Conf. and Workshop on Embryonic and Fetal Antigens in Cancer* (DTIE, AEC, Oak Ridge, Tenn., to be published).
- Ambrose, K. R., Anderson, N. G., and Coggin, jun., J. H., *Nature* (in the press).
- Hellström, I., Hellström, K. E., Pierce, G. E., and Yang, J. P. S., *Nature*, **220**, 1352 (1968).
- Tevethia, S. S., Crouch, N. A., Melnick, J. L., and Rapp, F., *Intern. J. Cancer*, **5**, 176 (1970).
- Dierlam, P. J., Anderson, N. G., and Coggin, jun., J. H., *Proc. First Conf. and Workshop on Embryonic and Fetal Antigens in Cancer* (DTIE, AEC, Oak Ridge, Tenn., to be published).
- Morton, D. L., Eilber, F. R., Joseph, W. L., Wood, W. C., Trahan, E., and Ketcham, A. S., *Ann. Surg.*, **172**, 740 (1970).

the aid of the very accurate observations from the Hewitt cameras at Malvern and Edinburgh is yielding information on the variations of the zonal wind speed, which is likely to depend on local time as well as latitude. Results from Cosmos 307 rocket¹² and Cosmos 316 (unpublished data) suggest that the rotation rate sometimes departs greatly from its average value. This conclusion is in accord with recent measurements of very strong neutral winds, especially in auroral regions at times of geomagnetic disturbance when winds of more than 500 m s⁻¹ have been recorded^{13,14}.

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- ¹ King-Hele, D. G., *Theory of Satellite Orbits in an Atmosphere* (Butterworths, London, 1964).
- ² King-Hele, D. G., Scott, D. W., and Walker, D. M. C., *Planet. Space Sci.*, **18**, 1433 (1970).
- ³ King-Hele, D. G., and Allan, R. R., *Space Sci. Rev.*, **6**, 248 (1966).
- ⁴ Challinor, R. A., *Planet. Space Sci.*, **17**, 1097 (1969); **18**, 1485 (1970).
- ⁵ Cole, K. D., *Planet. Space Sci.*, **19**, 59 (1971).
- ⁶ Rishbeth, H., *Planet. Space Sci.*, **19**, 357 (1971).
- ⁷ Weinstein, D. H., and Keeney, J., *Nature*, **231**, 109 (1971).
- ⁸ Isakov, M. N., Morosov, S. K., and Schnoll, E. E., COSPAR Meeting, Seattle, June 1971.
- ⁹ Volland, H., *Nature*, **232**, 248 (1971).
- ¹⁰ Gooding, R. H., *Nature Phys. Sci.*, **231**, 168 (1971).
- ¹¹ King-Hele, D. G., *Royal Aircraft Establishment Technical Report*, 71171 (1971).
- ¹² Hiller, H., *Royal Aircraft Establishment Technical Report*, 71151 (1971).
- ¹³ Rees, D., *J. Brit. Interplanet. Soc.*, **24**, 233 (1971).
- ¹⁴ Feess, W. A., COSPAR meeting, Seattle, June 1971.

Use of Astronomical Telescopes to measure Aerosol Pollution

ASTRONOMICAL observatories are particularly suited for studies of the background aerosol content of the atmosphere for four reasons. (1) The observatories are usually in remote locations away from the lights and smoke of cities. (2) Most observatories have standard equipment designed to correct the measured light fluxes of stars for the loss of brightness in the passage through the atmosphere (in other words, to correct for atmospheric extinction). (3) There is good global coverage of observatories. (4) Observations of the extinction of starlight by the atmosphere have been made for nearly forty years and are available for re-analysis. The last point is particularly

important because there are no other reliable records of the condition of the atmosphere in remote locations.

In this article we compare data from three astronomical observatories¹ with other measurements of the scattering of light by aerosols at clean air locations. The scattering coefficients obtained by the different methods support other evidence for the existence of a background level of aerosols in the atmosphere, and also establish the astronomical telescope as a potentially valuable tool for monitoring aerosol pollution.

Extinction measurements are made photoelectrically by observing the apparent brightness of standard stars at different zenith angles². Charlson *et al.*¹ have examined data from seven observatories for atmospheric extinction, but data that is statistically meaningful has so far only been obtained for three observatories.

The three observatories are Rattlesnake Ridge (Washington), Kitt Peak National Observatory (Arizona), and Cerro Tololo Interamerican Observatory (Chile). Rattlesnake Ridge Observatory is about 30 km west of the nearest centre of population, Richland, which has almost no polluting industry, and is at an altitude of 1,080 m in a clean, nearly desert, climate. Kitt Peak is at 2,064 m in a desert climate about 75 km west of the nearest centre of population (Tucson), and Cerro Tololo, by far the least polluted site, is at an altitude of 2,195 m about 450 km north of the nearest centre of population, Santiago.

The third column of Table 1 shows the geometric mean of the ratio of the extinction coefficient due to scattering from the total column of air between the observatory and the star ($\bar{b}_{\text{scat}}$) to the Rayleigh scattering by air (b_{Rayleigh}). These values have been interpolated to a wavelength of 500 nm to standard temperature and pressure. Absorption was neglected because it is expected to be very small compared with scattering. Charlson *et al.*¹ reported extinction k in magnitudes at the zenith so the following conversion was used to obtain the scattering coefficient $\bar{b}_{\text{scat}}$

$$\bar{b}_{\text{scat}} = \frac{0.92 k}{X}$$

where X is the optical depth for scattering above the observatory (≈ 8 km for a sea level observatory). The extinction due to ozone absorption in the Choppuis band was removed from this data assuming an optical depth for ozone of 0.25 cm at Cerro Tololo and 0.35 cm at the other sites. The super and subscripts in Table 1 are the 90% confidence limits. Rattlesnake Ridge showed much more variation than Kitt Peak or Cerro Tololo. These ratios are consistent with nephelometer and sunphotometer measurements at similar locations giving ratios of 1.5 on Mt Olympus, Washington, and 1.95 at Point Barrow, Alaska³.

Table 1 Light Extinction Properties of the Atmosphere over Three Observatories

Location (altitude)	Effective wavelengths	$\bar{b}_{\text{scat}}/b_{\text{Rayleigh}}$ at 500 nm (No. of nights measured)	$\bar{\alpha}_{RV}$	α_{total} $\bar{\alpha}_{VB}$	$\bar{\alpha}_{BU}$	$\bar{\alpha}_{RV}$	Aerosol only α (aerosol) $\bar{\alpha}_{VB}$	$\bar{\alpha}_{BU}$
Rattlesnake Ridge, Washington (1,080 m)	R ~ 693 nm V ~ 537 nm B ~ 440 nm U ~ 365 nm	2.0 +0.1 -0.9 (20 nights)	3.8 +1.4 -1.8	2.1 +1.6 -0.8	3.3 +0.9 -0.7	3.6 +1.4 -3.0	0.6 +3.2 -1.9	1.4 +3.1 -1.6
Kitt Peak, Arizona (2,064 m)	R ~ 693 nm V ~ 537 nm B ~ 440 nm U ~ 365 nm	1.7 +0.5 -0.3 (24 nights)	3.4 +1.7 -1.5	2.2 +0.7 -0.5	3.8 +0.2 -1.4	2.9 +2.6 -1.9	-0.4 +0.8 -3.0	—
Cerro Tololo, Chile (2,195 m)	V ~ 553 nm B ~ 440 nm U ~ 366 nm	1.3 +0.4 -0.3 (17 nights)	—	3.8 +1.2 -1.9	4.1 +0.8 -0.7	—	1.5 +3.5 -3.0	3.2 +3.3 -1.3

The astronomical measurements were made at several different wavelengths (see Table 1) that were defined using broad band (100 nm) glass filters. The wavelength dependence of the atmospheric scattering coefficient due to both aerosol and Rayleigh scatter can be described by

$$\bar{b}_{\text{scat}} \propto \lambda^{-\alpha_{\text{total}}}$$

where α_{total} is an empirically determined exponent having a value 4 in pure air. Similarly, the contribution to extinction from aerosol scatter alone can be described by

$$\bar{b}_{\text{scat}}(\text{aerosol}) \propto \lambda^{-\alpha(\text{aerosol})}$$

where $\alpha(\text{aerosol})$ has a typical value of 1.3 for urban air. Volz has related $\alpha(\text{aerosol})$ to size distributions of the form

$$\frac{dN}{d \log r} = Cr^{-\beta}$$

where dN is the number of particles in the logarithmic radius increment $d \log r$, C is a function of the total amount of aerosol and β is an exponent of about 3 for urban air.

Within a range of $2 < \beta < 6$ the following relationship applies

$$\beta = \alpha(\text{aerosol}) + 2$$

Table 1 lists the geometric mean values of α_{total} and $\alpha(\text{aerosol})$ obtained from the total extinction coefficients at the indicated wavelengths. For example, α_{RV} is measured between the red (693 nm) and the visible (537 nm for Cerro Tololo), and similarly for the other α s with subscript R implying "red", B implying "blue", and U implying "ultraviolet". The effective wavelengths for each site are shown in Table 1.

These wavelengths, incidentally, correspond to those used in the multiwavelength nephelometer results of ref. 4, although of course the telescope determinations refer to the entire line of sight whereas the nephelometer gives a measurement at a point.

In the rest of Table 1 the aerosol contribution has been isolated using the aerosol scattering coefficient $\bar{b}_{\text{scat}}(\text{aerosol}) = \bar{b}_{\text{scat}} - b_{\text{Rayleigh}}$. In some cases negative values of $\alpha(\text{aerosol})$ are obtained, when the extinction due to the aerosol component increases rather than decreases with increasing wavelength, and indeed more than half the nights at all three locations showed anomalous, that is, negative, values. This type of scattering behaviour has also been observed by Quenzel *et al.*⁵ in clean oceanic air during the Meteor Expedition. This type of scattering must be due to an aerosol size distribution different from the simple power law with β between 2 and 6. On very rare occasions anomalous aerosol extinction may even dominate the strong Rayleigh scattering wavelength dependence (once in a blue Moon). Because of the wide variation in the $\alpha(\text{aerosol})$ at all three locations, much more data will be required to characterize the wavelength dependence of background aerosol.

All the data reported¹ were reduced completely by computer from original raw intensity measurements. The probable error of a single reduced intensity measurement is typically less than 2% at 500 nm. This uncertainty increases slightly toward shorter wavelength. Because the extinction typical at an observatory of altitude 4,000 foot is only about 20% at this wavelength, it is possible for the extinction measurements to be uncertain by $\pm 10\%$. As the actual measurement error is usually less than 1%, we can credit most of the uncertainty to the fact that the standard stars are measured at random times and random zenith angles throughout the night, and are reduced by assuming no time or spatial variation in extinction during the night. Both time and spatial variation often do occur, however, due to unobserved clouds. As a $\pm 10\%$ error in extinction is related to only a 2% error in the intensity of the star at that wavelength, this is sufficient for most routine astronomical photometry. Better extinction measurements (a 1% error in extinction) could be obtained in the future by using a local intensity standard (such as a radioactive light source) so

that one measurement in space and time could be used to calculate the extinction of the light from a standard star. In calculating the wavelength dependence, the uncertainty in extinction exerts a strong influence on α , especially when $\Delta \log b_{\text{scat}}$ is very small. In the same way a slightly incorrect choice of effective wavelengths of the filters also exerts an influence on α . The determination of the effective wavelength for the broad band filters is at present complicated by the fact that the standard stars observed usually have different spectral distributions, usually with significant slopes in the wavelength region being measured, and include many different patterns of absorption lines. Using the mean values of $\bar{b}_{\text{scat}}$ shown in Table 1 the error in $\bar{b}_{\text{scat}}$ and wavelength can combine to make as much as a 60% error in the measured α s.

We conclude that astronomically determined measurements of atmospheric extinction are consistent with other measurements in clean air locations. These data provide information that can be used to characterize the background aerosol in the atmosphere and changes due to world-wide air pollution. So far, not enough old data has been reduced to establish possible secular changes in global atmospheric extinction, although Hodge⁶ has found a significant increase in atmospheric extinction over the Mt Wilson observatory in California in the past fifty years.

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- ¹ Charlson, R. J., Hodge, P. W., Lucke, P. B., Mannery, E. J., and Snow, T. P., *Project ASTRA Publication No. 2* (Univ. Washington, Seattle, 1970).
- ² Hardie, R. H., in *Astronomical Techniques* (edit. by Hiltner, W. A., (Univ. Chicago Press, Chicago, 1962).
- ³ Porch, W. M., Charlson, R. J., and Radke, L. F., *Science*, **170**, 315 (1970).
- ⁴ Ahlquist, N. C., and Charlson, R. J., *Atmospheric Environment*, **3**, 551 (1969).
- ⁵ Quenzel, H., *J. Geophys. Res.*, **75**, 2915 (1970).
- ⁶ Hodge, P. W., *Project ASTRA Publication No. 4* (Univ. Washington, Seattle, 1970); *Nature*, **229**, 549 (1971).

Exchange of SO₂ between the Atmosphere and Natural Waters

LITTLE is known about the rate of exchange of gas between the atmosphere and natural waters (such as oceans, rivers, lakes and airborne water droplets), and most work so far has concentrated on the more common atmospheric constituents, N₂, O₂, CO₂ and water vapour. The transfer of trace components, such as oxides of sulphur and nitrogen, has received almost no attention. This article is an attempt to predict the principal factors controlling the exchange of SO₂ across air-water interfaces in the natural environment.

Gas exchange rates are often given either as an exchange constant (k = flux of gas/concentration difference), or its reciprocal, which measures the "resistance" of the interface to gas transfer. Danckwerts¹ has shown that the overall resistance of a gas-liquid interface may be interpreted as the sum of the resistances of the individual phases:

$$\frac{1}{K_1} = \frac{1}{k_1} + \frac{1}{H k_g} \quad (1)$$

$1/K_1$ is the total resistance on a liquid phase basis, $1/k_1$ and $1/k_g$ are the individual resistances of the liquid and gas phases and H is the Henry's law constant, where

$$H = \frac{\text{equilibrium concentration in gas phase}}{\text{equilibrium concentration of unionized dissolved gas in liquid phase}}$$

For a sparingly soluble gas, the liquid phase resistance term in equation (1) is much larger^{2,3} than that for the gas phase. This is partly a reflexion of the large value of H for such a gas, and partly due to the fact that the molecular diffusivity (and hence diffusive transport) of most gases is about two orders of magnitude less in water than it is in air. By contrast the exchange of water vapour, in common with evaporation-condensation of any pure liquid, is controlled by resistance in the gas phase. The behaviour of SO_2 is likely to fall between these two extremes. One reason for this is the moderate solubility of the gas in water, which makes the gas phase resistance term larger than for a less soluble gas. Another factor is that the value of k_1 may be increased because of the chemical reactivity of the gas in solution. Provided that the liquid is not too acidic, SO_2 molecules entering the water will be rapidly hydrated and then ionized. Transport of SO_2 near the liquid side of the interface may then be enhanced because the normal dissolved gas concentration gradient will be supplemented by the gradient of ionic species. Such enhancement of the value of k_1 for a chemically reactive gas has been observed by Hoover and Berkshire⁴ in laboratory studies using CO_2 . These authors have derived the following expressions for calculating the degree of enhancement to be expected.

$$\alpha k_{1(\text{inert})} = k_{1(\text{reactive})} \quad (2)$$

where

$$\alpha = \frac{\tau}{(\tau-1) + \left\{ \tanh \left[\left(\frac{k^* \tau}{D} \right)^{\frac{1}{2}} \frac{D}{k_{1(\text{inert})}} \right] / \left(\frac{k^* \tau}{D} \right)^{\frac{1}{2}} \frac{D}{k_{1(\text{inert})}} \right\}} \quad (3)$$

α is the ratio of the k_1 values for a reactive and an inert gas exchanging under identical conditions, τ is the ratio of total to ionic forms of the gas in solution, k^* is the hydration rate constant for the gas, and D is the molecular diffusivity of the dissolved gas molecules.

Although derived for CO_2 exchange, equations (2) and (3) should also be applicable to SO_2 transfer. As the hydration rate constant for SO_2 ($\sim 10^6 \text{ s}^{-1}$) is much higher than that for CO_2 ($\sim 10^{-2} \text{ s}^{-1}$) the exchange enhancement for SO_2 should be considerably more pronounced than the enhancement found for CO_2 .

If equations (1) to (3) are to be used to estimate exchange rates for SO_2 under environmental conditions, typical values must be found for $k_{g(\text{SO}_2)}$ and $k_{1(\text{inert})}$. The value for $k_{1(\text{inert})}$ must then be multiplied by the appropriate value of α to obtain $k_{1(\text{SO}_2)}$. The value of α depends critically on τ , which in turn is a function of the pH of the water. To cover most environmental situations, I shall calculate α for pH values 2 to 9.

Using the data given by Schooley⁵, a mean value for $k_{g(\text{H}_2\text{O})}$ over the oceans of about $3,000 \text{ cm h}^{-1}$ may be derived. Values over smaller bodies of water (lakes, rivers and so on) will almost certainly be less than this, but a value of $3,000 \text{ cm h}^{-1}$ will be assumed for the present. It will further be assumed that k_g for SO_2 and H_2O are approximately equal. A typical value for $k_{1(\text{inert})}$ seems to be more difficult to obtain. Downing⁶ gives values between 0.4 and 7.5 cm h^{-1} for O_2 in various freshwater environments although, exceptionally, values as high as 300 cm h^{-1} have been recorded. Measurements at sea lie in the region 4 to 36 cm h^{-1} (my work to be published). A reasonable guess would seem to be about 10 cm h^{-1}

for $k_{1(\text{inert})}$. It will be shown that the values chosen for $k_{g(\text{SO}_2)}$ and $k_{1(\text{inert})}$ are not very critical to the conclusions to be drawn from the calculations.

Table 1 shows the results of the calculations in which it is assumed that $H = 3.8 \times 10^{-2}$ (ref. 7), $D = 2 \times 10^{-5} \text{ cm}^2 \text{ s}^{-1}$, $k^* = 3.4 \times 10^6 \text{ s}^{-1}$ (ref. 8).

Table 1 Calculation of the Ratio of the Gas to the Liquid Phase Resistance

pH	α	$k_{1(\text{SO}_2)}$ cm h^{-1}	$\frac{1}{k_{1(\text{SO}_2)}} = r_1$ $\text{h cm}^{-1} \times 10^5$	$\frac{1}{H k_{g(\text{SO}_2)}} = r_g$ $\text{h cm}^{-1} \times 10^3$	r_g/r_1
2	2.7	27	3,700	8.8	0.24
2.8	11.7	117	855	8.8	1.03
3	18.0	180	560	8.8	1.57
4	169	1,690	59	8.8	14.92
5	1,376	13,760	7.3	8.8	120.6
6	2,884	28,840	3.5	8.8	251.4
7	2,966	29,660	3.4	8.8	258.8
8	2,967	29,670	3.4	8.8	258.8
9	2,967	29,670	3.4	8.8	258.8

The final column in Table 1 gives the ratio of the gas to the liquid phase resistance. At a pH of 2.8 both resistances are approximately equal but when the pH is below this value the liquid phase resistance is more important than the resistance of the gas phase. Above a pH of 2.8 the gas phase resistance

becomes increasingly dominant. The exchange of SO_2 is interesting because it changes from liquid to gas phase control as the pH is raised. By contrast, although the r_1 value for CO_2 decreases with increasing pH (due to the ionic gradient effect) it is always greater than the value of r_g . Thus exchange of CO_2 is principally controlled by the resistance of the liquid phase.

For a number of years chemical engineers have been making measurements of SO_2 transfer rates in packed columns^{9,10}. Their experimental results seem to support the ideas outlined above, in that both the gas and liquid phase resistances appear to exert an appreciable effect on the overall resistance.

The pH of most natural waters in surface environments is between 4 and 9. It can therefore be concluded from the calculations set out above that, for almost all environmental air-water interfaces, gas phase resistance should control the SO_2 exchange rate. This conclusion would not have been substantially affected by using rather different values for $k_{g(\text{SO}_2)}$ and $k_{1(\text{inert})}$. Under calm conditions in both air and water, $k_{g(\text{SO}_2)}$ and $k_{1(\text{inert})}$ might be $1,000 \text{ cm h}^{-1}$ and 2 cm h^{-1} respectively; in this case r_g and r_1 would have been equal at a pH of about 3.0. By contrast, under turbulent conditions in both phases ($k_{g(\text{SO}_2)} = 5,000 \text{ cm h}^{-1}$, $k_{1(\text{inert})} = 30 \text{ cm h}^{-1}$), r_g and r_1 become equal when the pH is about 2.5. Thus for a wide range of mixing conditions, pH values greater than about 4 imply that gas rather than liquid phase resistance will control the exchange of SO_2 . The behaviour of SO_2 in crossing a natural air-water interface will therefore tend to resemble that of water vapour rather than O_2 or CO_2 .

Two tentative conclusions may be drawn from this result. First, the vertical gradients of SO_2 and water vapour (humidity

deficit) close to the sea surface should be similar. Second, the mean residence time of an SO_2 molecule in the atmosphere should be of the same order as that for a water molecule (~ 10 days, ref. 11). This contrasts with the much larger residence time for a CO_2 molecule (~ 7 yr, ref. 12); in this case, exchange is controlled by resistance in the liquid phase. A residence time of about 10 days for SO_2 is in reasonable agreement with the mean value of 5 days given by Junge¹¹.

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- ¹ Danckwerts, P. V., *Gas-Liquid Reactions*, 145 (McGraw-Hill, London, 1970).
- ² Bolin, B., *Tellus*, **12**, 274 (1960).
- ³ Skirrow, G., in *Chemical Oceanography* (edit. by Riley, J. P., and Skirrow, G.), **1**, 227 (Academic Press, London, 1965).
- ⁴ Hoover, T. E., and Berkshire, D. C., *J. Geophys. Res.*, **74**, 456 (1969).
- ⁵ Schooley, A. H., *J. Mar. Res.*, **27**, 335 (1969).
- ⁶ Downing, A. L., in *River Pollution* (edit. by Klein, L.), **2**, 224 (Butterworths, London, 1962).
- ⁷ Terraglio, F. P., and Manganelli, R. M., *J. Air Pollut. Control Assoc.*, **17**, 403 (1967).
- ⁸ Eigen, M., Kustin, K., and Maass, G., *Z. Phys. Chem.*, **30**, 130 (1961).
- ⁹ Adams, F. W., *Ind. Eng. Chem.*, **25**, 424 (1933).
- ¹⁰ Whitney, R. P., and Vivian, J. E., *Chem. Eng. Prog.*, **45**, 323 (1949).
- ¹¹ Junge, C. E., *Air Chemistry and Radioactivity*, **3** (Academic Press, London, 1963).
- ¹² Craig, H., *Tellus*, **9**, 1 (1957).

Determination of the Density of Seawater

KREMLING has recently described¹ a promising new method for the determination of the density of seawater. The agreement he demonstrates between his results and those based on the equation of state, determined by Knudsen as a function of salinity and temperature, is even better than he suggests. This arises from an error in Kremling's interpretation of the new definition of salinity.

Salinity, S , is now defined by the International Tables² in terms of conductivity. The best estimate of chlorinity³ is obtained by using the relation $\text{Cl} = S/1.80655$ and not the Knudsen equation. One thus arrives at a lower value for Cl than estimated by Kremling which is most marked for the lower values of Cl . When the densities are recalculated one finds that Kremling's estimates are reduced by 0.20 at $S = 5\%$ and by 0.009 at $S = 20\%$ with linear interpolation between. Application of this correction suggests complete agreement in the mean with Knudsen and exemplifies once again the great quality of standards measurements in the early years of the century.

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- ¹ Kremling, K., *Nature*, **229**, 109 (1971).
- ² *International Oceanographic Tables* (National Institute of Oceanography of Great Britain and UNESCO, 1966).
- ³ Cox, R. A., Culkln, F., and Riley, J. P., *Deep-Sea Res.*, **14**, 203 (1967).

Structure of Fibrous Carbon

THE existence of fibrous or whisker carbon has been reported in both synthetic studies of graphite^{1,2} and in naturally occurring graphite³. The synthetic studies involved decomposition of hydrocarbons over metals¹ and decomposition of carbon monoxide over iron² and, particularly in the second of these studies, the occurrence of metal catalyst within the fibre was observed. Furthermore, it has been established¹ that the average orientation of the carbon crystallites is with their (0002) orientation parallel to the axis of the fibre. In this communication we describe the use of high resolution electron microscopy to establish the precise relationship between the orientation of the graphite planes and the catalyst particle within the fibre.

A nickel foil was heated to 700° C in 600 mm Hg of methane or propane for several hours and the ensuing film of graphite was stripped from the foil surface by immersion in concentrated HCl. The film of graphite was sufficiently thin to be studied by transmission electron microscopy and it contained outcrops of fibrous carbon as well as polycrystalline and high quality graphitic carbon. Fig. 1A shows the appearance of a typical carbon fibre formed in an atmosphere of methane, the very high width to length ratio of the fibres formed in methane being characteristic of this hydrocarbon. Fig. 1B is an enlargement of part of the previous figure so that the (0002) lattice planes can be seen. There are three principal regions of the fibre: in the centre is an irregular particle of what is believed to be metal (in this case it would be nickel) and surrounding this is a highly crystalline carbon with the (0002) lattice planes parallel to the surface of the metal particle; on the periphery of the fibre there is a narrow region of amorphous carbon. The first point of interest is the close parallelism of the graphite layer planes with the surface of the metal particle, because it has been reported

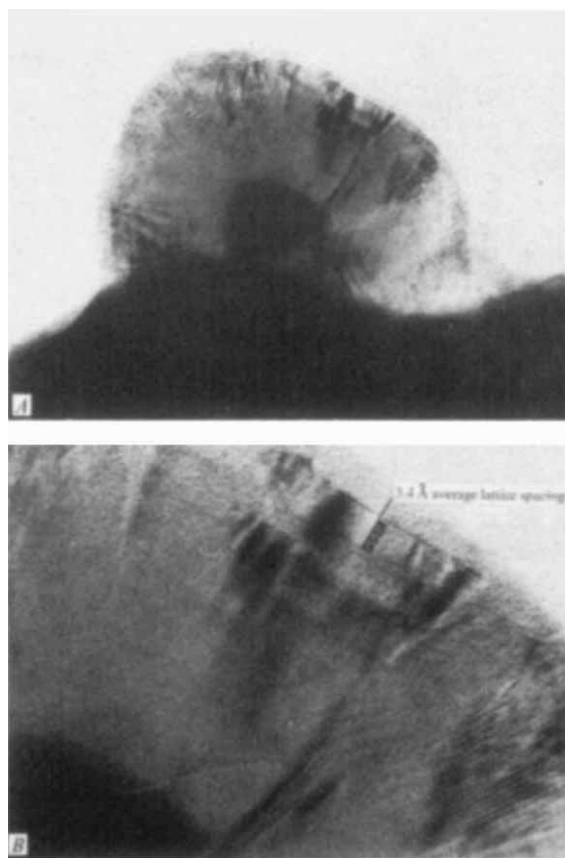


Fig. 1 A, Fibre produced by methane pyrolysis over nickel ($\times 312,000$); B, Enlargement of a region of Fig. 1A, with (0002) lattice planes delineated for identification ($\times 1,000,000$).

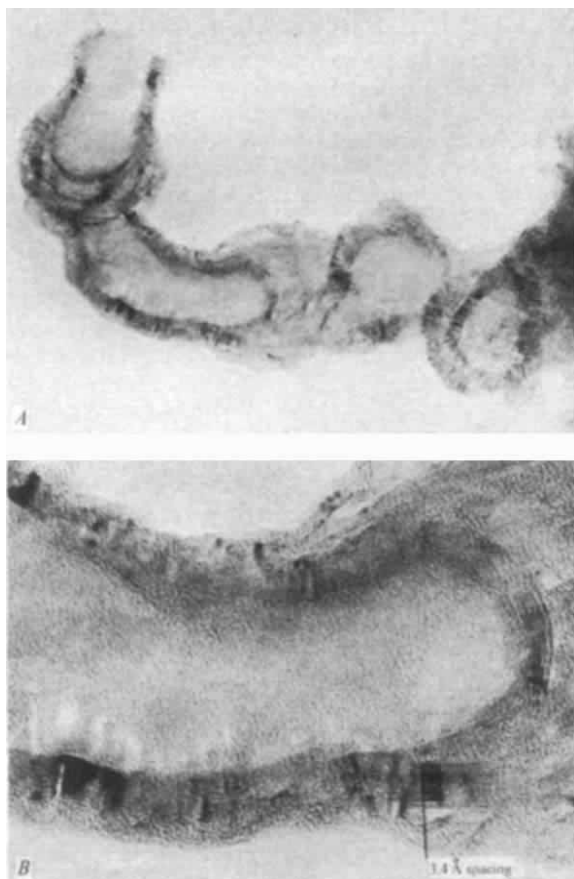


Fig. 2 *A*, Fibre produced by propane pyrolysis over nickel ($\times 312,000$); *B*, Enlargement of a region of Fig. 2*A* with (0002) lattice planes delineated for identification ($\times 1,000,000$).

that there is a preference for graphite growth on the (110) faces of nickel⁴. The irregular shape of the metal particle precludes the exposure of any single lattice plane all around its surface and yet there are no marked irregularities in the carbon fibre that would suggest a preferred direction of growth. From the intensity variations in the original micrograph, we also conclude that this fibre is approximately hemispherical in shape.

Fig. 2*A* is a carbon fibre formed by propane pyrolysis. No metal particle was observed in this fibre but the open nature of the end of the fibre and possibly along the centre indicates that the metal was removed by the preparative treatment in concentrated HCl. Fig. 2*B* is an enlargement of part of this fibre. Areas of resolved (0002) graphite lattice planes are again distinguishable throughout the fibre even at its root where it has grown from the main carbon deposit. The overall shape of this fibre, however, is markedly different from fibres produced by methane and the arcing that occurs periodically along the central region of the fibre suggests that the direction of growth bends in and out of the plane of the micrograph. The average direction of the (0002) lattice planes is, however, parallel to the axis of the fibre or, to be more precise, the direction is parallel to the areas where metal particles were believed to have been situated. The difference between these fibres and those produced from methane could be explained by the mobility of the metal particle in the case of the propane fibres; a much longer and more distorted fibre would then be produced as the metal moved away from the main carbon deposit.

The structure of these fibres—in particular, that produced by methane pyrolysis—raises several questions about the mechanism of their formation. It is difficult to imagine that the metal could exert catalytic influence up to 300 Å away from the metal surface and yet it is equally difficult to explain a mechanism that requires diffusion of active species perpendicular

to the layer planes of graphite. Furthermore the micrograph in Fig. 1 shows that where there are faults in the layer plane structure which might provide an easy channel for diffusion, there is no marked production of carbon at the end of these channels as would be expected if a diffusion mechanism predominated. This argument is equally valid whether active species are considered to diffuse out or hydrocarbon molecules are considered to diffuse in, thus depositing the carbon layers adjacent to the metal surface and building up the fibre from the inside. We suggest that, as each carbon layer is laid down, atomically dispersed nickel is incorporated within this layer. In this form, catalytic deposition could take place on the outer layers of the growing fibre and would continue for some distance away from the metal although there would be a dilution of the metal atoms by the subsequent layers laid down. If this mechanism is applicable to general graphite formation, a dilution of nickel would be reached at some distance away from the metal surface where it is no longer possible for laminar graphite to be laid down; this has been observed by ourselves and by other workers⁵. At this stage the crystallite size of the carbon that is deposited is very small, and the crystallites are randomly oriented with respect to each other. This mechanism is further supported by oxidation studies on pyrolytic graphites that have been laid down by this technique. After removal of the graphite by oxidation there remain agglomerates of nickel that have been dispersed within the graphite¹, the agglomeration obviously being caused by the heating necessary for the oxidation reaction because agglomerates were not observed in the unreacted material.

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¹ Robertson, S. D., *Carbon*, **8**, 365 (1970).

² Ruston, W. R., Warzee, M., Hennaut, J., and Waty, J., *Carbon*, **7**, 47 (1969).

³ Patel, A. R., and Deshpande, S. B., *Carbon*, **8**, 242 (1970).

⁴ Presland, A. E. B., Roscoe, C., and Walker, P. L., jun., *Third Conference on Industrial Carbons and Graphite*, London, 1970.

⁵ Blau, G., and Presland, A. E. B., *Third Conference on Industrial Carbons and Graphite*, London, 1970.

BIOLOGICAL SCIENCES

Requirement of Thymus-dependent Lymphocytes for Potentiation by Adjuvants of Antibody Formation

THE mode of action of immunological adjuvants is of theoretical as well as practical importance. Adjuvants can not only increase immune responses; they can also bring about change from one type of immune response to another. For example, doses of bovine serum albumin (BSA) that in the absence of adjuvant induce tolerance, in the presence of adjuvants induce antibody formation^{1,2}. To understand how adjuvants switch on antibody formation it may be necessary to determine which of the cells involved in the immune response are affected by adjuvants. Earlier experiments³⁻⁵ led to the conclusion

that the cells initially involved in the action of adjuvants are macrophages. This interpretation was based on observations that particulate adjuvants (such as bacteria) are ingested by macrophages but not by lymphocytes. Non-particulate or particulate adjuvants taken up by macrophages in culture and injected into syngeneic mice increased the antibody response of the recipients to two proteins (*Maia squinada* haemocyanin or bovine serum albumin (BSA)); in contrast, adjuvants taken up by lymphoid cells used to reconstitute immune responses

adjuvant (FCA-ICFA with 100 µg of *Mycobacterium butyricum*/ml.) or 5 mg of 'Retinol' in ICFA were injected intraperitoneally 1 h after administration of antigen in saline. Antibody was assayed by the Farr test^{1,4}. The antigen-binding capacity for each group of six mice (except for those marked with an asterisk, which contained 5) is presented as the geometric mean of the antigen-binding capacity (ABC) expressed as log₁₀, together with the standard error (s.e.) calculated from the variance.

Table 1 Serum Antibody against Bovine Serum Albumin in Normal and T Cell-Deprived Mice with and without Adjuvants

Treatment	Restoration	Adjuvant	Primary ABC	s.e.	Secondary ABC	s.e.
None	—	—	-0.42	0.14	0.57	0.15
None	—	LPS	0.34	0.08	1.61	0.17
None	—	Pertussis	0.24	0.06	1.73	0.26
None	—	ICFA	0.38	0.14	1.32	0.19
None	—	CFA	0.83	0.18	1.62	0.23
None*	—	'Retinol'	1.23	0.22	2.17	0.26
TX, ALS	—	—	-1.04	0.12	-0.96	0.12
TX, ALS*	—	LPS	-1.15	0.23	-1.12	0.18
TX, ALS	—	Pertussis	-1.32	0.14	-0.83	0.25
TX, ALS	—	ICFA	-1.37	0.11	-0.37	0.12
TX, ALS	—	CFA	-1.22	0.06	-0.93	0.15
TX, ALS	—	'Retinol'	-1.49	0.13	-0.82	0.21
TX, X-ray	Marrow	—	-1.36	0.19	-1.06	0.09
TX, X-ray	Marrow	LPS	-1.19	0.17	-1.27	0.13
TX, X-ray	Marrow	Pertussis	-1.08	0.08	-1.56	0.24
TX, X-ray	Marrow, thymus	—	-0.22	0.15	0.21	0.10
TX, X-ray	Marrow, thymus	LPS	0.13	0.09	1.26	0.08
TX, X-ray*	Marrow, thymus	Pertussis	0.27	0.06	1.34	0.27

in irradiated recipients had no demonstrable effect on antibody formation.

It is now clear that immune responses to many antigens require co-operation of thymus-dependent (T) and bone marrow derived (B) lymphocytes⁶, the former possibly functioning as an antigen-concentrating system while the latter give rise to cells which can synthesize and release antibody^{7,8}. If it is accepted that adjuvants act initially on macrophages, which are not themselves immunocompetent, two hypotheses can be considered. According to the first, adjuvants increase the probability of direct interaction of macrophages containing antigen and B lymphocytes capable of producing antibody, by-passing the T lymphocytes. According to the second, adjuvants increase the effectiveness of the interaction of macrophages and T lymphocytes, and this results in greater stimulation of the appropriate B lymphocytes to produce specific antibody. In an attempt to decide between these hypotheses we have compared the effects of adjuvants on antibody production against BSA in normal young adult CBA male mice and CBA mice deprived of T lymphocytes by neonatal thymectomy followed by two injections at 5 and 6 weeks of 0.2 ml. of rabbit anti-mouse lymphocyte serum (ALS) in some experiments, or thymectomy at 8 weeks followed by 850 r. whole-body irradiation and reconstitution with syngeneic bone marrow⁹. As an additional control, thymectomized (TX), irradiated and reconstituted mice receiving also a syngeneic thymus graft under the renal capsule¹⁰ have been used.

The primary immunization was by intraperitoneal inoculation with 1 mg centrifuged BSA in 0.5 ml. saline; 20 days later the mice were bled for determination of primary antibody responses. The same day they were injected with 100 µg of BSA intraperitoneally, and blood samples were taken 10 days later for determination of secondary antibody responses. As adjuvants 2 µg of purified *E. coli* lipopolysaccharide (LPS) or 0.4 µg of *Bordetella pertussis* vaccine (Wellcome) were administered together with the antigen in saline. Freund's incomplete adjuvant (ICFA—mineral oil, Bayo F, containing mannoside—mono-oleate, Difco), or Freund's complete

Representative results are shown in Table 1. In mice depleted of T cells by either procedure antibody responses to BSA are decreased, as previously described by Taylor¹¹, and the disparity between normal and T cell-deprived mice is greater when the primary immunization is made with adjuvant. In mice reconstituted with a thymus graft marked stimulation of antibody formation by adjuvants is again seen.

It therefore seems that T lymphocytes are required for potentiation of antibody formation against BSA by adjuvants. It has been suggested³⁻⁵ that one of the effects of adjuvants may be to bring about the release from macrophages of a factor which stimulates proliferation of lymphocytes, and that when antigen is presented at this time there is an increased probability of antibody formation rather than tolerance induction. In keeping with this, it has been found¹² that vitamin A, which is an efficient adjuvant but is not in itself immunogenic, produces marked blast transformation and proliferation of cells in the thymus-dependent areas of draining lymph nodes, whereas substances, such as paraffin oil, without adjuvant activity have at most slight effects on the thymus-dependent areas of lymph nodes. These findings are consistent with the possibility that one of the effects of adjuvants is to increase the effectiveness of the interaction of macrophages and T lymphocytes. The macrophages concentrate antigen, which is presented to T lymphocytes in highly immunogenic form along with a stimulus for the latter to proliferate. This greatly increases the probability that T lymphocytes will mount an immune response, which in turn allows them to co-operate in antibody production by B lymphocytes. But whether or not the interaction of macrophages with T cells is augmented by adjuvants it seems that stimulation of T cells is an important aspect of the mechanism of action of at least some adjuvants. It is of interest to note that injections of allogeneic cells may also provide a general stimulus to T cell proliferation and overcome the requirement for co-operation by populations of T cells specifically stimulated by antigen in the potentiation of an immune response¹³.

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- ¹ Mitchison, N. A., *Proc. Roy. Soc., B*, **161**, 275 (1964).
- ² Dresser, D. W., and Mitchison, N. A., *Adv. Immunol.*, **8**, 129 (1968).
- ³ Unanue, E. R., Askonas, B. A., and Allison, A. C., *J. Immunol.*, **103**, 71 (1969).
- ⁴ Spitznagel, J. K., and Allison, A. C., *J. Immunol.*, **104**, 119 (1970).
- ⁵ Spitznagel, J. K., and Allison, A. C., *J. Immunol.*, **104**, 128 (1970).
- ⁶ Roitt, I. M., Greaves, M. F., Rorrigiani, G., Brostoff, J., and Playfair, J. H. L., *Lancet*, ii, 367 (1969).
- ⁷ Mitchell, G. F., and Miller, J. F. A. P., *J. Exp. Med.*, **128**, 821 (1968).
- ⁸ Mitchison, N. A., in *Sixth Intern. Immunopathol. Symp.* (edit. by Miescher, P.) (Schwabe, Basel, 1970).
- ⁹ Davies, A. J. S., Leuchars, E., Wallis, V., and Koller, P. C., *Transplantation*, **4**, 438 (1966).
- ¹⁰ Leuchars, E., Cross, A. M., and Dukor, P., *Transplantation*, **3**, 28 (1965).
- ¹¹ Taylor, R. B., *Transplant. Rev.*, **1**, 114 (1969).
- ¹² Taub, R. N., Krantz, A. R., and Dresser, D. W., *Immunology*, **18**, 171 (1970).
- ¹³ Benacerraf, B., in *Sixth Intern. Immunopathol. Symp.* (edit. by Miescher, P.) (Schwabe, Basel, 1970).

Variations of the Expression of HL-A Antigens on Human Diploid Fibroblasts *in vitro*

ALL kinds of human nucleated cells so far tested express on their surface the HL-A antigens which form the major histocompatibility system in man¹. These antigens, which are easily detected on lymphocytes and on platelets of peripheral blood, have also been found on other human cells²⁻⁹. In long term human cell cultures, it has been possible to demonstrate the remarkable stability of the HL-A antigens. Analogous observations have been reported in mouse regarding the antigens controlled by H-2 histocompatibility system¹⁰. We report here results obtained from the study of ten varieties of human fibroblasts, which enabled us to analyse the behaviour of the antigens of the HL-A system in cell cultures of limited duration¹¹.

Taking advantage of skin graft experiments performed in our laboratory¹², we obtained fibroblasts through standard procedures, from skin biopsies of healthy volunteers belonging to families whose tissues had been extensively and repeatedly typed in lymphocytotoxicity and complement fixation on platelets (Table 1).

Fibroblasts were typed using Terasaki's lymphocytotoxicity technique, slightly modified¹³. The dose of immune sera was increased, the fibroblast suspension was adjusted to 1,000 cells per test, and the reading was made by dye exclusion.

The antisera used (Table 2) came from either multiparous women, immunized or multiple transfused individuals. Each specificity was defined by one to five reagents. All the results obtained with the reagents defining a same specificity were concordant.

Each specificity detected was tested directly against either the corresponding antiserum, or a cross-reacting antiserum¹⁴: for example, anti-HL-A1, which recognized HL-A3, and HL-A11 (refs. 15, 16), anti-HL-A5 which recognizes Da6, Da20, Da24, Te18 (ref. 17), anti-HL-A7 which recognizes BB (15). Except with fibroblast 109' (Table 1), the fibroblast

specificities detected were always the same as those determined in corresponding lymphocytes^{8,9}. The HL-A antigens progressively disappeared (Table 3). The loss of expression of these antigens did not seem to be random, and occurred by locus, and not by haplotype. In nine cases out of ten, the antigens controlled at the second locus were the first to disappear.

Table 1 Comparison of the HL-A Antigens detected on the Lymphocytes and the Fibroblasts from a Same Donor

Donors	HL-A genotypes of donors of lymphocytes and fibroblasts
32	HL-A2, BB ^a /HL-A9, HL-A5
35	HL-A9, HL-A5/HL-A3 ^d , Da20 ^b
46	HL-A10, HL-A7/HL-A3 ^d , —
212	HL-A3 ^a , HL-A7/HL-A10, BB ^a
GUI . . .	HL-A2, HL-A7/HL-A3, Da6 ^b
33	HL-A9, HL-A5/HL-A11 ^c , Da18 ^c
115	HL-A2, Te18 ^b /HL-A3, Da24 ^b
154	HL-A2, —/HL-A2, AA ^c
44 ^f	HL-A2, —/HL-A12 ^c
109 ^g	HL-A2, —/HL-A1, HL-A8
109 ^g	(HL-A7) (HL-A1) (HL-A5)

In each case, the formulae show the genes at the two haplotypes, which determine the corresponding antigens on the lymphocytes.

—, Genes, the products of which are not serologically detected. Italic types show the products of the genes which have been serologically found on fibroblasts. a, BB antigens, not determined directly, positive reaction with anti-HL-A7 due to the cross-reaction BB, HL-A7. b, Antigens Da20, Da6, Te18, Da24, not determined directly for shortage of available antisera, positive reaction with anti-HL-A5 due to the cross-reaction between the former specificities and HL-A5 antigen. c, Antigen HL-A11, not tested directly, positive with anti-HL-A1, due to cross-reaction HL-A11, HL-A1. d, Antigen HL-A3, tested directly by anti-HL-A3, but positive reaction with anti-HL-A1, due to cross-reaction between the specificities HL-A3, HL-A1. e, Antigens Da18, AA, HL-A12, specificities not tested for shortage of available antisera. f, Genotype not determined. g, Complete disparity between the groups determined on lymphocytes 109 and fibroblasts 109'.

Table 2 Immune Sera used for the Determination of HL-A Antigens on Fibroblasts and Lymphocytes

Specificities	Antisera		Doses used (μl.)			
			On fibroblasts		On lymphocytes	
First locus						
HL-A2	Hisl.	Multiple-transfused	5	3	1	
	Mari.	Multiparous	3	1	0.06	
	2.12.260	Multiparous	5	3	0.5	
	27.11.317	Multiparous	5	3	0.5	
HL-A9	Eluate		0.06	0.03	0.015	0.015
	Dup.	Multiple-transfused	0.25		0.03	0.015
HL-A1	Gius.	Multiple-transfused	0.06	0.03	0.03	0.015
	110	Multiparous	0.12	0.06	0.12	0.06
	Hayer	Multiple-transfused	0.25	0.12	0.25	0.12
HL-A3	Eluate		0.12	0.06	0.06	0.03
HL-A10	Loui.	Immunized	5	3	0.25	
	Gran.	Multiple-transfused	0.25		0.06	
Second locus						
HL-A5	Plati.	Multiple-transfused	0.5	0.25	0.06	0.03
	Goud.	Multiple-transfused	0.5	0.25	0.06	0.03
	4.12.401	Multiparous	5	3	0.5	
	9.12.017	Multiparous	5	3	0.5	
HL-A7	Eluate		0.10	0.06	0.015	
	Eluate		0.2	0.1	0.06	0.03
	25.11.262	Multiparous	5	3	0.5	
	Barr.	Multiple-transfused	0.5	0.25	0.12	0.6
	Fitou		1	0.3	0.16	0.1

Table 3 Time of Disappearance of HL-A Antigens on Fibroblasts in Culture

Approximate time scale	Strains of fibroblasts	32 _h		33 _h		44 _h ^F		GUI . . . _h		46 _h		212		35 _h		109 ^g _h		154		115	
		Initially detected antigens		First locus	Second locus	First locus	Second locus	First locus	Second locus	First locus	Second locus	First locus	Second locus	First locus	Second locus	First locus	Second locus	First locus	Second locus	First locus	Second locus
		Number of passages	HL-A2 HL-A9 BB ^a HL-A5	HL-A9 HL-A11 ^c HL-A5 Da18 ^e	HL-A2 HL-A12	HL-A2 HL-A3 HL-A7 Da6 ^b	HL-A10 HL-A3 ^d HL-A7 —	HL-A3 ^e HL-A10 HL-A7 BB ^a	HL-A9 HL-A3 ^e HL-A5 Da20 ^b	HL-A1 — HL-A7 HL-A5	HL-A2 HL-A2 — AA ^e	HL-A2 HL-A3 Te18 ^b Da24 ^b									
		Passage																			
Two months	20	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	21	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	22	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	23	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	24	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	25	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	26	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	27	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	28	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	29	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Two months	30	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	31	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	32	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	33	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	34	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	35	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	36	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	37	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	38	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	39	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Six months	40	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	41	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	42	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	43	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	44	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
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66	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
67	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
68	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	

+, Passages when antigens were found. —, Disappearance either of specificity or of a cross-reaction demonstrating this specificity. a, b, c, d, e, f, g, see Table 1. h, Six fibroblasts were studied for the first time at a passage number not exactly determined (between fifteen and twenty-five—estimated at twenty in the table). i, Antigen HL-A3 recognized by an anti-HL-A1 anti-serum, because of the cross-reaction HL-A3, HL-A1 ;+ without i indicates the persistency of a positive reaction with anti-HL-A3 and the negatization of the reaction with anti-HL-A1. ○, No degeneration. ●, Total degeneration.

In three cases—fibroblasts 46, 212, 35 (Table 3)—the HL-A3 antigen was recognized both by anti-HL-A3 immune serum, and by the anti-HL-A1 reagent, and we could observe that the serological negatization of the cross-reaction preceded that of the direct reaction.

The disappearance of histocompatibility antigens occurred together with the senescence of the cultures and preceded cell degeneration and death. The presence of all the HL-A antigens on the fibroblasts coincided with a phase of normal cellular multiplication with a generation time of about 24 h. In six cases out of ten, the partial disappearance of the antigens controlled by the second locus occurred after a number of passages which varied with the strains of the fibroblasts studied, and corresponded to a phase of an apparently normal cellular multiplication. There was, however, an increasing generation time from 24 h to 96 h and sometimes even more. On the contrary, in four cases out of ten, though the cells survived after the disappearance of all the HL-A antigens, the multiplication stopped rapidly, the morphological aspect of the culture was that of a complete degeneration of the strain, rapidly followed by death. Degeneration could be diagnosed by a decrease in the cell number and morphologically by the presence on the cells of aggregates of cytoplasmic origin, and by the loss of their initial spindle-shaped oblong form.

Concordance has already been observed on lymphocytes, and on fibroblasts regarding certain given groups. Our study confirms the presence of HL-A antigens on the surface of fibroblasts, in spite of their lesser sensitivity to immunological lysis, probably in relation with a lesser concentration of antigenic sites. It implies the concept of a large distribution of antigens on human tissues, and underlines the importance of their role in transplantation. Our results differ from those of other authors⁸ on the stability of the HL-A antigens on human fibroblasts in culture: more specificities of the second locus have been observed and for a longer time.

As we have explained, the serological behaviour of the products, which are controlled by the first and the second loci, is different when these products are studied in the course of time on cultured human diploid fibroblasts. By loss of specificity we mean the disappearance at a given moment of a specificity which could previously be detected in a reproducible manner. Our experimental conditions do not allow us to determine whether it is a question of qualitative or quantitative loss of antigens, but absorption experiments are in progress. It is possible that antigens are still present, but can no longer be detected by immune sera. In nine out of ten fibroblast strains, however, the products controlled by the second locus were the first to become serologically undetectable. It is unlikely that the antisera used to determine the antigens at the first locus are more sensitive than those applied to the antigens of the second locus, for comparison of the titres of the immune sera shows that the mean activity is the same in the two cases. It thus seems that the stability of the antigens controlled by the first and second HL-A loci is different, and that the products of the former are more stable in our experimental conditions. It is still possible that at a certain stage of cell degeneration following multiple passages, some of these antigens may become more susceptible to trypsin action, because of a general change in the cell membranes. The asymmetry observed between the products controlled by the first and second HL-A loci can be compared with that observed between the D and K regions of mouse H-2 locus, shown *in vitro* by the mixed lymphocyte test¹⁸, and *in vivo* by graft versus host reaction¹⁹. Furthermore, cross-reactions are a general phenomenon observed in the serology of the HL-A system¹⁴; this experiment, which shows that the loss of cross-reactivity is independent of that of direct reactivity, suggests that the antigenic determinants responsible for the direct specificity and those responsible for cross-reactions are distinct.

Finally, our results lead us to discuss the possible biological role of the HL-A system: in four cases out of ten, either degeneration or cell death (fibroblasts 32, 33, 44, 35) rapidly followed

once the point was reached at which specificity in the HL-A system was no longer detectable. On the other hand, in the other six cases (fibroblasts GUI, 46, 212, 154, 109', and 115), where at least one of the specificities controlled by the two loci of the HL-A system was still detected, no sign of cellular senescence was observed except an increased generation time. Indeed, from the results obtained, it is not possible to confirm that there is a relationship between loss of HL-A antigens and cell degeneration. But if these results are compared with those obtained for the mouse H-2 locus in which all the strains have at least one set of antigens of the H-2 locus²⁰⁻²³, it becomes tempting to speculate that HL-A antigens behave in the same fashion. The practical interest of the detection of HL-A antigens is first their role as genetic markers, yet with a certain limitation regarding their stability of expression during the cell senescence period; and second, the fact that it makes it possible to define the identity of strains and to determine their age.

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- ¹ Dausset, J., Colombani, J., Legrand, L., and Fellous, M., *Histocompatibility Testing*, 53 (Munksgaard, Copenhagen, 1970).
- ² Yunis, E. J., Yunis, J. J., and Brand, R. G., *Blood*, **25**, 1 (1965).
- ³ Metzgar, R. S., Flanagan, J. F., and Zmijewski, C. A., *J. Immunol.*, **95**, 494 (1965).
- ⁴ Milgrom, F., and Kano, F., *Histocompatibility Testing*, 179 (Munksgaard, Copenhagen, 1965).
- ⁵ Papermaster, V. M., Papermaster, W. B., and Moore, G. E., *Fed. Proc.*, **28**, 379 (1969).
- ⁶ Engelfriet, C. P., Heersche, J. N. M., Eijssvoogel, V. R., and Van Loghem, J. J., *Vox Sang.*, **11**, 625 (1966).
- ⁷ Melief, C. J. M., van der Hart, M. A., Engelfriet, C. D., Eijssvoogel, V. P., and van Loghem, J. J., *Vox Sang.*, **15**, 187 (1968).
- ⁸ Miggiano, V. C., Nabholz, M., and Bodmer, W. F., *Histocompatibility Testing*, 623 (Munksgaard, Copenhagen, 1970).
- ⁹ Thorsby, E., and Lie, S., *Vox Sang.*, **15**, 44 (1968).
- ¹⁰ Klein, D., Merchant, D. J., Klein, J., and Shreffler, D. C., *J. Nat. Cancer Inst.*, **44**, 11 (1970).
- ¹¹ Hayflick, L., *Exp. Cell Res.*, **37**, 611 (1965).
- ¹² Dausset, J., Rapaport, F. T., Legrand, L., Colombani, J., and Marcelli-Barge, A., *Histocompatibility Testing*, 381 (Munksgaard, Copenhagen, 1970).
- ¹³ Terasaki, P. I., and McClelland, J. D., *Nature*, **204**, 998 (1964).
- ¹⁴ Colombani, J., Colombani, M., and Dausset, J., *Histocompatibility Testing*, 79 (Munksgaard, Copenhagen, 1970).
- ¹⁵ Sveigaard, A., *Ser. Haematol.*, **2**, 3 (1969).
- ¹⁶ Thorsby, E., and Kissmeyer-Nielsen, F., *Vox Sang.*, **17**, 102 (1969).
- ¹⁷ Colombani, M., Colombani, J., Dastot, H., Mayer, S., Tongio, M., and Dausset, J., *Rev. Franc. Etud. Clin. Biol.*, **14**, 995 (1969).
- ¹⁸ Rychlikova, M., Demant, P., and Ivanyi, P., *Folia Biol.*, **16**, 3, 218 (1970).
- ¹⁹ Demant, P., *Folia Biol.*, **16**, 4, 273 (1970).
- ²⁰ Klein, E., *Nobel Symp.* (1965).
- ²¹ Klein, E., and Moller, E., *J. Nat. Cancer Inst.*, **31**, 347 (1963).
- ²² Moller, E., *J. Nat. Cancer Inst.*, **33**, 979 (1964).
- ²³ Cann, H. M., and Herzenberg, L. A., *J. Exp. Med.*, **117**, 267 (1963).

Fluorescent Y Screening of Hospitalized Newborns

QUINACRINE dihydrochloride and quinacrine mustard have been used to identify chromosomes at metaphase^{1,2} and the Y chromosome in interphase³⁻⁶. We now report a preliminary survey of blood smears for Y chromosome fluorescence from all

Table 1 Characteristics of the Infants Studied

Case	Gestation (weeks)	Birth weight (g)	Admission diagnosis	Y chromosome	Congenital defects	Final outcome
1	35	1,680	Umbilical omphalocele Respiratory distress	XYX	Mongoloid facies Umbilical omphalocele Cong. bilat. hydronephrosis VSD PDA	Died age 2 days
2	40	3,630	Suborbital dermoid	Large	Frontal encephalocele Bilat. choanal atresia	Alive Surgical correction
3	31	1,340	Prematurity (twin of case 4)	Small	Nil	Alive
4	31	1,845	Prematurity (twin of case 3)	Small	Nil	Alive
5	40	2,400	Cleft lip and palate	Small	Bilat. cleft lip and palate Undescended testis	Alive
6	41	2,780	Ambiguous genitalia	Normal	Hypospadias Imperforate anus	Alive Colostomy
7	36	1,770	Prematurity Ambiguous genitalia	Normal	Marked hypospadias	Alive

newborn males transferred to this hospital from February to April 1971.

Blood smears were air-dried, fixed for 5 min in 100% methanol, dried, stained in 0.5% quinacrine dihydrochloride ('Atebrine', Gurr) for 5 min, rinsed in running tap water, dried, mounted in phosphate buffer (pH 4.5) and examined under a Zeiss photomicroscope with an HBO 200 lamp, excitor filter BG 12 and barrier filter 53/44.

Blood smears were taken from 140 male and five female infants. Ten lymphocytes and usually ten neutrophils were examined from each smear without prior knowledge of the sex or clinical status of the infant. Y chromosome abnormalities or normal variants, confirmed by karyotypes, were detected in five infants by this fluorescence study (Table 1 and Fig. 1). A further two infants, admitted specifically for definitive sex determination because of ambiguous genitalia, had normal male fluorescence patterns and karyotypes (Table 1 and Fig. 1).

In the XYX smear (case 1), three cells with two fluorescent spots (F-spots) were seen in the initial twenty leucocytes. On

further screening, approximately 30% of 100 cells examined had two F-spots, and none with a single large F-spot. F-spots were noticed to be very large in almost all leucocytes from case 2 (large Y chromosomes).

Smears from cases 3, 4 and 5 had definite F-spots but these were so small and difficult to see that they might have been classified as negative (female). Smears from the five females had no F-spots and were easily identified.

Two cells in each of two other smears contained two F-spots and were shown to be false positives by karyotypes. In the remaining smears, 83% of lymphocytes (and 65% of neutrophils) contained an F-spot of normal size (Fig. 1, cases 6, 7). Karyotypes were not made on any of these males.

This study shows that Y chromosome abnormalities and variants can be rapidly detected by this fluorescence technique and then confirmed by karyotype. Parallel chromosome studies were not done and the number of false negatives is unknown. This study might suggest that the number of false negatives is small because a large proportion of cases with interesting Y chromosomes were found. This was, however, a selected population, hospitalized because of neonatal problems.

Whether the large proportion of interesting Y chromosome cases was related to selection of this type of patient or was a chance finding will have to be determined by further studies.

Identification of male nuclear sex by the fluorescent technique is a valuable addition to the sex chromatin test in cases of ambiguous genitalia.

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- Caspersson, T., Farber, S., Foley, G. E., Kudynowski, J., Modest, E. J., Simonsson, E., Wagh, U., and Zech, L., *Exp. Cell Res.*, **49**, 219 (1968).
- Caspersson, T., Zech, L., Modest, E. J., Foley, G. E., Wagh, U., and Simonsson, E., *Exp. Cell Res.*, **58**, 141 (1969).
- Zech, L., *Exp. Cell Res.*, **58**, 463 (1969).
- Pearson, P. K., Bobrow, M., and Vosa, C. G., *Nature*, **226**, 78 (1970).
- Polani, P. E., and Mutton, D. E., *Brit. Med. J.*, **1**, 138 (1971).
- Conen, P. E., Lewin, P. K., and Vakil, D., *Canad. Med. Assoc. J.*, **104**, 925 (1971).

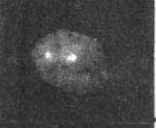
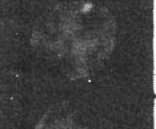
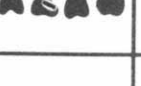
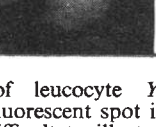
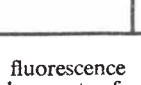
CASE	Y CHROMOSOME	LEUKOCYTE	KARYOTYPE	
			G(21-22)	Y
1	XYX			
2	LARGE			
3,4,5	SMALL			
6,7	NORMAL			

Fig. 1 Comparison of leucocyte Y fluorescence pattern with karyotype. The fluorescent spot in leucocytes from cases 3-5 was present but difficult to illustrate because of its small size.

Scrapie in Immunologically Deficient Mice

ALTHOUGH the agent responsible for scrapie is classified as a slow virus, many features distinguish it from a typical virus¹⁻³, one of which is its apparent failure to elicit an immune response. Attempts to demonstrate antibody by neutralization^{4,5}, complement fixation⁴, precipitation⁴ and immunofluorescence^{4,6} have been unsuccessful, but the role of cell-mediated immunity in scrapie has been less well studied.

Neonatal thymectomy did not alter the incubation period or pathology in one small series of mice inoculated intracerebrally with scrapie². We have studied the course of scrapie produced by intraperitoneal inoculation in thymectomized mice, lethally irradiated and reconstituted with foetal liver cells. Although they were markedly depleted of thymus-derived (T) lymphocytes and were incapable of rejecting allogeneic skin grafts, their response to the scrapie agent was not different from that of normal mice.

CBA/He mice of both sexes were used: group I was thymectomized at 4 weeks old, irradiated with 900 r. and reconstituted with syngeneic foetal liver cells; group II received 900 r. and was reconstituted with foetal liver cells; group III was untreated. Four weeks later they all received skin grafts from A strain donors and after 9 days half the animals in each group were inoculated intraperitoneally with 0.25 ml. of 10⁻¹ dilution of normal BSVS mouse brain; the rest received 0.25 ml. of 10⁻¹ dilution of brain from BSVS mice with scrapie; these materials were supplied by Dr R. L. Chandler.

Cell-mediated immune function was followed by assessment of skin allograft survival. In addition the percentage of cells bearing the θ allo-antigen (a marker for T lymphocytes⁷) and the microscopic appearance of the lymphoid organs were studied in two animals from each group, 7 months after inoculation (Table 1). Animals in group I retained skin allografts throughout the experiment, had very few θ -bearing cells in their blood and were markedly depleted of lymphocytes in the thymus-dependent areas of spleen and lymph nodes⁸. In contrast, mice in groups II and III rejected their skin grafts after 13.6 and 10.3 days respectively, had normal numbers of θ -bearing blood lymphocytes and normal lymphoid organs.

Mice were observed five or six times a week for the appearance of scrapie following the criteria of Dickenson *et al.*⁹. No mice inoculated with normal brain developed signs of scrapie, but those injected with scrapie-affected brain developed scrapie and either were killed in extremis or died within 230 days of inoculation. Many of the immunologically deficient mice in group I died before developing signs of scrapie. None of these mice had neuropathological lesions consistent with scrapie during the clinical phase of the disease.

Table 2 shows that scrapie began between 163 and 170 days in all three groups. There was no significant difference between mean times of death of 190 and 205 days seen in groups I

Table 2 Time Course of Scrapie

Group	No. of mice	Onset of disease (days)	Time from inoculation to death (days)	
			Range	Mean
I	7	163-170	174-200	190
II	16	163-170	200-223	215
III	24	163-170	173-228	205

and III respectively; they were even closer than appears because four of the mice in group I were killed when judged to be pre-terminal in order to obtain satisfactorily histology and peripheral blood for determination of θ -bearing lymphocytes. Although the extent and magnitude of the nervous system lesions were not compared in detail, the neuropathological changes revealed by haematoxylin and eosin or Cajal were consistent with scrapie in all three groups. Thus we could demonstrate no appreciable difference in the clinical and pathological aspects of scrapie in immunologically deficient mice depleted of T lymphocytes when compared with normal mice. This suggests that cell-mediated immunity neither contributes to the pathogenesis of scrapie nor plays a significant role in the host's defence against the agent.

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- ¹ Latarjet, R., Muel, B., Haig, D. A., Clarke, M. D., and Alper, T., *Nature*, **227**, 1341 (1970).
- ² Gibbons, R. A., and Hunter, G. D., *Nature*, **215**, 1041 (1967).
- ³ Field, E. J., *Intern. Rev. Exp. Pathol.*, **8**, 129 (1969).
- ⁴ Gibbs, J. G., Gajdusek, C. C., and Morris, J. A., *Public Health Service Publication No. 1378*, 195 (1965).
- ⁵ Haig, D. A., and Clarke, M. C., *Public Health Service Publication No. 1378*, 215 (1965).
- ⁶ Chandler, R. L., *Vet. Rec.*, **71**, 38 (1959).
- ⁷ Raff, M. D., *Nature*, **224**, 378 (1969).
- ⁸ Parrott, D. M. V., De Sousa, M. A. B., and East, J., *J. Exp. Med.*, **123**, 191 (1966).
- ⁹ Dickinson, A. G., Meikle, V. M. H., and Fraser, H., *J. Comp. Pathol.*, **78**, 293 (1968).

Extraction of Prostaglandins from Human Blood

EXISTING methods for extracting prostaglandins from tissues or seminal plasma with 70-90% ethanol¹ or acetone² give poor and inconsistent recoveries from blood. Equilibrium dialysis and recovery experiments with labelled and unlabelled prostaglandins show that this is due to binding with precipitated serum albumen (unpublished results of W. G. U.). Plasma globulins and intact blood cells do not seem to interact with the prostaglandins. When precipitation of protein is avoided by using 40-50% ethanol acidified with formic acid, the prostaglandins can be extracted quantitatively into chloroform. The method is as follows: (1) centrifuge heparinized or citrated blood; (2) mix 1 volume each of plasma and saline with 2 volumes of ethanol (analytical grade); (3) extract twice with petrol (boiling point 40°-60° C, 2 x 2 volumes) to remove neutral fats including carotene; (4) adjust to pH 3-3.5 with formic acid (1-3% v/v); (5) extract twice with amounts of chloroform each equal to the total volume in stage 4; (6) evaporate chloroform in a thin film evaporator at 30° C; (7) redissolve residue in 10 ml. chloroform and evaporate to aid the removal of formic acid; (8) blow oxygen-free nitrogen through

Table 1 Cellular Immune Status

Group	Treatment	Skin allograft survival in days (mean)	% θ -bearing cells in blood*	Lymphoid* organs
I	Thymectomy 900 r. Reconstituted with foetal liver	Greater than 200	4, 17	Lymphocyte depletion in in thymus-dependent areas
II	900 r. Reconstituted with foetal liver	9-19 (13.6)	77, 68	Normal
III	None	9-14 (10.3)	74, 73	Normal

* Evaluated after 7 months.

vessel until odour of formic acid is gone; (9) dissolve in Krebs solution for bioassay or in appropriate solvent for chromatography.

The plasma should be either fresh or frozen (-10°C in the present experiments). Plasma stored in glass and freeze-dried whole plasma were found to contain acidic biologically active material which was not a prostaglandin. Formic acid is used in stage (4) to achieve the desired pH because its pK_a is near pH 3 and because its volatility allows easy removal; the amount of acid needed varies with different samples of plasma. Chloroform is used to extract the alcoholic solution (stage 5) since only a small amount of alcohol or water enters the chloroform phase; diethyl ether and ethyl acetate used in other procedures are miscible with 50% ethanol.

The resulting extract in stage (9) is still crude, and contains bilirubin, phospholipids and traces of neutral fats in addition to the prostaglandins. Substantial amounts of haematin will also be present if haemolysis has occurred. These impurities do not affect the biological assays but they can affect the rate at which prostaglandins run on thin-layer chromatography. Phospholipid material can be removed by precipitation in cold acetone^{3,4}, but this is undesirable because a biologically active unidentified material is formed probably from the phospholipid fraction. This might account for the high blood levels of prostaglandin reported by Hickler⁴. The extracts can, however, be purified suitably for thin-layer chromatography by evaporating the chloroform, dissolving the residue in 10 ml. of 70% ethanol and shaking briefly with 250–500 mg Fuller's earth. After diluting the supernatant to 50% with water and acidifying with formic acid, the prostaglandins re-extract quantitatively into chloroform. This procedure removes haematin and phospholipids, but not bilirubin.

Assays of PGE₁, E₂, F_{1a} and F_{2a} on the rat fundus⁵ and of PGA₁ and A₂ in anaesthetized cats and rats^{6,7} gave recoveries of 102% (range 72–140%, 52 experiments) from human blood containing 0.01–1 µg/ml. added prostaglandin. The levels of prostaglandin activity in peripheral venous blood from fifteen normal males was found to be 1.6 ± 0.9 ng/ml. (assayed as prostaglandin E₂ ± s.d.).

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¹ Samuelsson, B., *Biochim. Biophys. Acta.*, **84**, 218 (1964).

² Ambache, N., *Biochem. Pharm.*, **12**, 421 (1963).

³ Hanahan, D. J., Dittner, J. C., and Warashina, E., *J. Biol. Chem.*, **228**, 685 (1957).

⁴ Hickler, R. B., *Prostaglandin Symp. of the Worcester Foundation for Experimental Biology* (edit. by Ramwell, P. W., and Shaw, J. E.), 279 (Interscience, New York, 1968).

⁵ Vane, J. R., *Brit. J. Pharmacol.*, **12**, 344 (1957).

⁶ Horton, E. W., and Jones, R. L., *J. Physiol.*, **200**, 56P (1969).

⁷ Crowshaw, K., McGiff, J. C., Strand, J. C., Lonigro, A. J., and Terragno, N. A., *Fed. Proc.*, **29**, 645 Abs. (1970).

New Porous Biomaterials by Replication of Echinoderm Skeletal Microstructures

AN important aspect of materials science is the development of new porous solids for prosthetic applications, especially for synthetic teeth and bone replacement. To be acceptable, such

biomaterials must meet some rather demanding specifications, among which are high strength and compatibility with body fluids or tissues. The structure of the porous medium is important as the pores must be both interconnected and have the proper size, shape and uniformity to permit ingrowth and attachment of body tissue. The problem lies in attaining the optimum combination of these properties to yield a high strength to weight ratio material, with a microstructure which best facilitates firm attachment of tissue. Ideally these materials should be easily machined or fashioned into particular shapes for different individual implants, and should be relatively inexpensive.

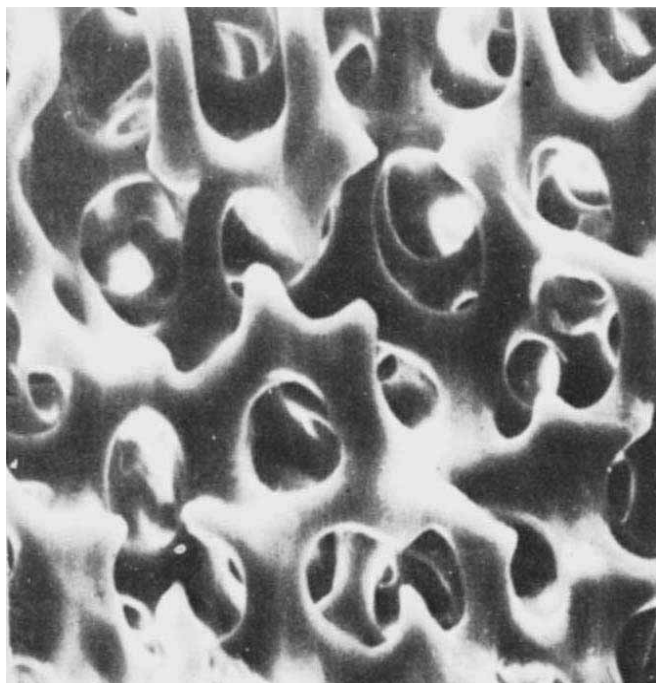


Fig. 1 Three dimensional fenestrate structure of skeletal high-magnesium calcite secreted by echinoderms. Photomicrograph by scanning electron microscopy. The diameter of the pores is approximately 10–15 µm

Ceramics show considerable promise in that strength and inertness requirements are likely to be met. Such materials have high compressive strength, do not corrode and have a specific gravity similar to that of bone^{1,2}. But because of the difficulties of creating the ideal pore structure artificially, we propose a new technique of replicating the microstructure found in the CaCO₃ skeletal elements of echinoderms. Using these methods it seems possible to select a natural microstructure with the most desirable geometry or morphology for a particular application, and then reproduce this structure by replacing the original skeletal material with one possessing adequate physical, chemical and mechanical properties.

The mineral phase of echinoderm ossicles is high-magnesium calcite³ which is secreted in optical continuity. Each spine of a sea urchin or echinoid, for example, is indistinguishable from a single crystal by X-ray diffraction or optical methods. The skeletal calcite, however, is permeated by interconnecting channels forming a fenestrate microstructure in spite of the single crystal nature of the inorganic phase (Fig. 1). In life, these passageways are filled with various organic cells and body fluids⁴. Donnay and Pawson⁵ have described this structure as a "periodic minimal surface" which divides space into two interpenetrating regions, each of which is a single yet multiple connected domain. The two regions, however, have no connexion with each other and the surface is the interface

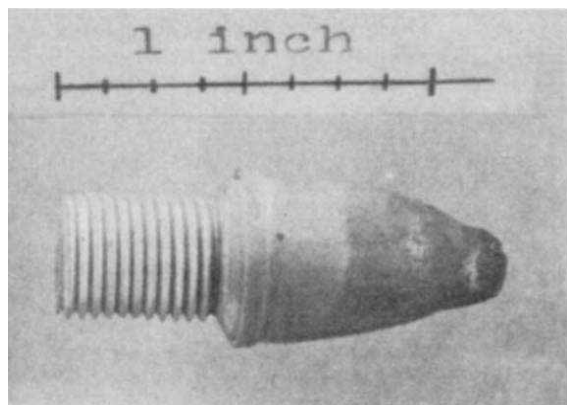


Fig. 2 Screw thread machined from a spine of the echinoid *Heterocentrotus trigonarius*.

between the skeletal calcite phase and the soft-tissue organic phase. Maximum contact for crystal growth is provided by this structure, which according to Donnay and Pawson⁵ has not been found in nature other than in echinoderm skeletons.

Although the fenestrate microstructure is found in almost all echinoderm skeletal ossicles⁵⁻⁷, few of these are large enough for practical applications. Spines of the echinoid *Heterocentrotus* are of sufficient size and ready availability to constitute a suitable source of material for replication. The genus *Heterocentrotus*, commonly referred to as the "slate-pencil urchin", is widely distributed and common in the Indo-Pacific area. The external morphology of the spines ranges from rather slender, pencil-like bodies with a tricarinate tip, to very thick, cylindrical or club-like forms. The length and width of the larger primary spines may exceed 160 and 13 mm respectively.

Although echinoderm skeletons are characterized by a unique and potentially useful microstructure, the calcium carbonate of which they consist is highly unsuitable for prosthetic applications. Calcite is considerably softer ($H=3$, Mohs scale) than the hydroxyapatite ($H=5$) found in bones

and teeth. Other undesirable properties of calcite include high solubility, perfect rhombohedral cleavage, and decomposition in the presence of acids. In spite of its high degree of cleavage, however, the high-magnesium calcite of echinoderm ossicles is easily machined without damage, as illustrated by the threaded echinoid spine in Fig. 2. The very properties that preclude the use of this mineral in human implants, however, facilitate reproduction of the microstructure in other materials.

Three dimensional negative replicas, made successfully from both epoxy resin and sodium silicate, reproduce the exact morphology of the void phase. Before vacuum impregnation, spines of *Heterocentrotus* are immersed in 5% sodium hypochlorite to remove organic matter, rinsed with distilled water and dried at 100° C. General Electric No. 3255 'Permafil', catalysed with 0.8% tertiary butyl perbenzoate, is used to prepare the epoxy resin replica. Air is removed from the pore volume in vacuum and replaced by the resin which effectively fills all pores. After curing the impregnated samples for 24 h at room temperature, the original calcite of the skeletal structure is removed by dilute (1 : 10) HCl. The same procedure is used to prepare the sodium silicate negative replicas except that the curing time is extended to 4 days. Epoxy and sodium silicate replicas are illustrated in Figs. 3 and 4.

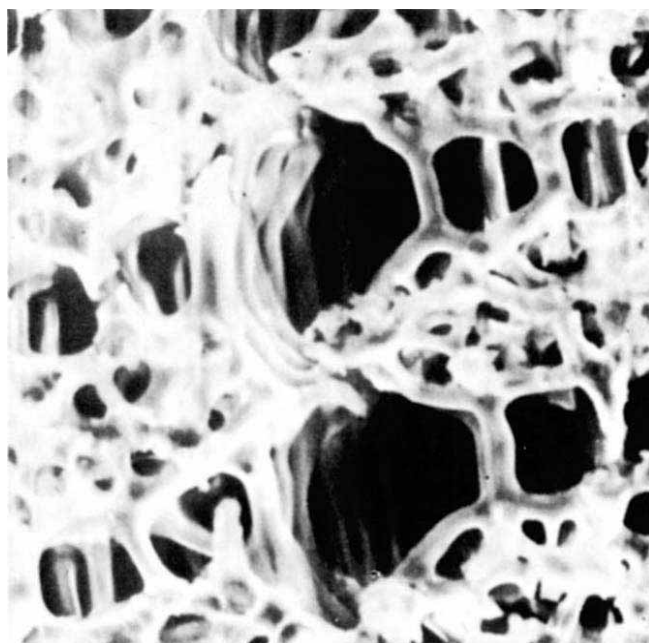


Fig. 4 Scanning electron photomicrograph of a sodium silicate negative replica of the microstructure from a spine of *Heterocentrotus trigonarius*.

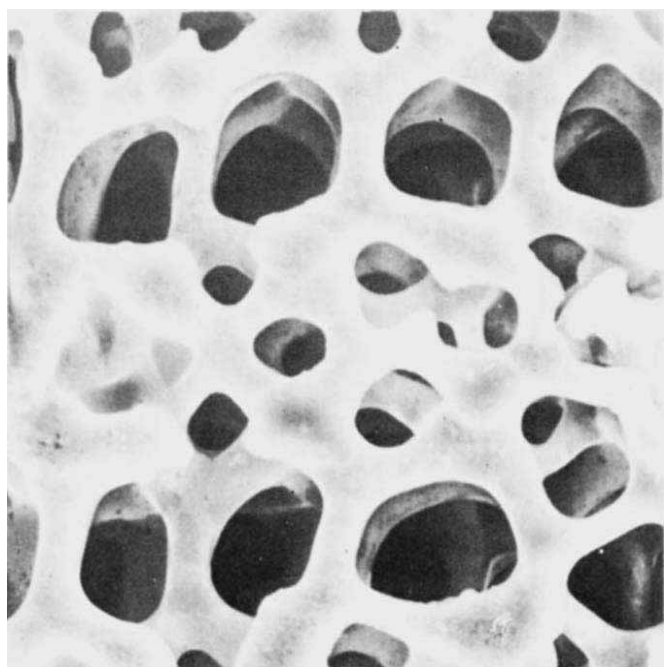


Fig. 3 Scanning electron photomicrograph of an epoxy resin negative replica of the microstructure from a spine of *Heterocentrotus trigonarius*.

Vacuum impregnation techniques can be used to prepare negative replicas of a periodic minimal surface, a structure with desirable geometric properties apparently unique to echinoderm skeletons. Positive replicas consisting of high strength ceramic materials could be made by impregnation of the primary negative replicas or by solid state reaction with the high-magnesium calcite. Phosphate-bonded alumina ceramic, for example, is particularly suitable in that it is well tolerated by tissues, does not elicit an immunologic response, and allows the attachment of fibrous connective tissue⁸. The concept of replicating natural microstructures for special applications offers considerable potential for the development of new porous biomaterials with an optimum combination of strength, density and infiltrability.

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¹ Smith, L., *Arch. Surg.*, **87**, 653 (1963).

² Klawitter, J. J., Hulbert, S. F., Talbert, C. D., and Fitts, C. T., in *Use of Ceramics in Surgical Implants* (edit. by Hulbert, S. F., and Young, F. A.) (Gordon and Breach, 1969).

³ Weber, J. N., *Amer. J. Sci.*, **267**, 537 (1969).

⁴ Pilkington, J. B., *J. Mar. Biol. Assoc. UK.*, **49**, 857 (1969).

⁵ Donnay, G., and Pawson, D. L., *Science*, **166**, 1147 (1969).

⁶ Nichols, D., and Currey, J. D., in *Cell Structure and its Interpretation* (edit. by McGee-Russell, S. M., and Ross, K. F. A.) (Arnold, London, 1968).

⁷ Weber, J. N., Greer, R. T., Voight, B., White, E. W., and Roy, R., *J. Ultrastructure Res.*, **26**, 355 (1969).

⁸ Bhaskar, S. N., Cutright, D. E., Knapp, M. J., Beasley, J. D., Perez, B., and Driskell, T. D., *Oral. Surg.*, **31**, 282 (1971).

Spontaneous Rhythms in Physiological Control Systems

MEAN arterial blood pressure in man undergoes spontaneous and broadly repetitive fluctuations with a typical period of about 10 s. We present an explanation for these fluctuations which we believe may also be applicable to the vasomotor activity associated with thermoregulation and therefore widely relevant.

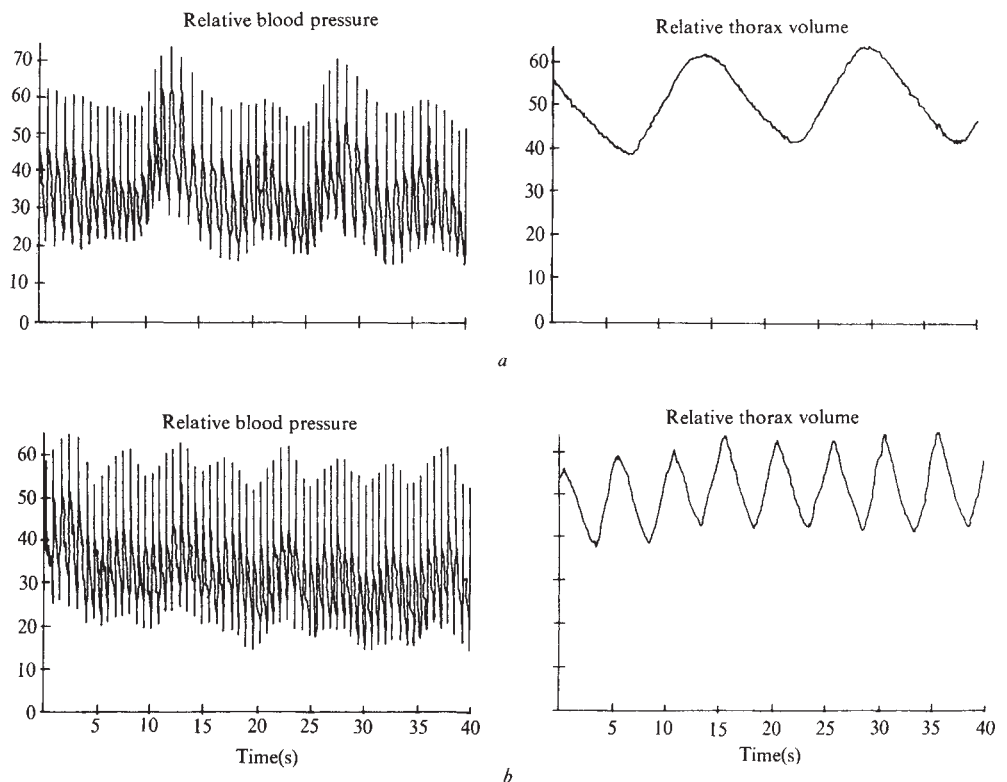
Fig. 1a illustrates the two broadly periodic fluctuations in the arterial blood pressure of a seated adult breathing voluntarily. One of these is related to respiration; the other occurs spontaneously, probably originates in vasomotor action, and is loosely described as a Traube wave which can also occur

during periods of apnoea¹. We believe that this spontaneous oscillation is intrinsic in the sino-aortic reflexes for regulating blood pressure, as a consequence of system nonlinearity, and that it is subject to entrainment² by the respiratory rhythm. The two oscillations can exist simultaneously provided that the respiratory period exceeds that of the spontaneous oscillation. But if the subject breathes at a higher frequency than that of the spontaneous oscillation, only the respiratory component remains, unless the respiration is too shallow to produce the effect (Fig. 1b). Detailed spectral analysis of the blood pressure confirms this observation.

The magnitude of the external signal, here respiration, required to entrain the spontaneous oscillation depends on the relative frequency of external signal and spontaneous oscillation and is much greater for signal frequencies appreciably below that of the spontaneous component than for frequencies in the region of, or even appreciably greater than, that of the spontaneous component. It has been possible to accommodate the physiological facts and observations in a model³ (Fig. 2a). Disturbances such as postural changes, or respiratory movements, can affect the system; respiratory disturbances are thought to contribute additively because the arteries in which the baroreceptors are located pass through the intrathoracic cavity. Because the arterial walls are elastic, variations in intrathoracic pressure produce redistributions in arterial blood volume, hence a change in distension of the blood vessels which in turn determine baroreceptor activity. Even in the absence of any disturbance, however, the model will continue to oscillate spontaneously.

With systems structured in this way, entrainment is known to be possible with adequate magnitudes of disturbance (Fig. 2a and b). The gain K_{NL} of the non-linear element to a sinusoidal signal input is inversely proportional to the signal amplitude, as far as the output component at the same frequency is concerned, because the output magnitude is fixed by the limits between which it switches. The overall gain round the feedback loop is $K_{NL} KG(j\omega)$; to meet the Nyquist criterion for stability the Nyquist plot of this loop gain must pass within the point $(-1,0)$ at ω_0 , the frequency of spontaneous oscillation. Fig. 2b shows the frequency response of the linear part $KG(j\omega)$ of this loop gain at various frequencies. Thus the

Fig. 1 a, A typical record of arterial blood pressure of a seated resting adult. The respiratory period is 15 s and there are two dominant low frequency components in the arterial pressure waveform, one of which has the same period as the respiration. **b**, The respiratory period is 5 s and a component of the same period only is evident in the arterial pressure waveform.



critical point of instability is reached when the value of $KG(j\omega_0)$, shown by X_0 , is equal to $-1/K_{NL}$; but the latter is under the control of an externally applied signal. Consequently if the magnitude $-1/K_{NL}$ is made greater than $KG(j\omega_0)$ by applying a sufficiently large external periodic disturbance (with frequency, ω_1 say) (Fig. 2a) spontaneous oscillation will cease and the system responds only to the disturbance. The magnitude of disturbance needed to produce this condition, entrainment, is proportional to the magnitude A_1 determined according to the graphical method of Johnson⁵, which evaluates the vector difference $[X_1 - X_0]$ or $K[G(j\omega_1) - G(j\omega_0)]$. If the frequency response KG of the linear elements circles in towards the origin at higher frequencies as shown (this is broadly true for vascular smooth muscle and other biological effectors), the magnitude of the disturbance required to produce entrainment is much larger for disturbance frequencies (ω_1 , say) appreciably below the frequency of spontaneous oscillation than for frequencies (ω_2 , say) near, or even appreciably above, that of the spontaneous oscillation (compare A_1 with A_2).

We consider that this property of "selective entrainment" will account for our experimental observations. As another consequence of its nonlinear nature, the model can simulate the high degree of control accuracy of the blood pressure control system. For example, in the normal adult, a positive Valsalva manoeuvre leading to a 60% reduction in cardiac output (which in the absence of nervous regulation would produce a corresponding decrease in mean arterial blood pressure) may often produce only about 5% decrease in mean blood pressure⁶. Similarly, the output of the model will deviate by only 3% from the reference when the corresponding effect is simulated (this is done by decreasing the parameter K by 60%). It would not be possible to achieve this degree of accuracy if the nonlinear element were replaced by a linear

block because of the stability restrictions defined by the Nyquist criterion⁷.

This raises the possibility that a similar mechanism applies in other biological control systems—especially those with a high degree of control accuracy and periodic oscillations in the controlled variable. This can be tested by looking for "selective entrainment" of the spontaneous component by a periodic disturbance introduced as close as possible to the sensors of the system. We have applied this technique in an investigation of the human thermoregulatory system. Burton⁸ has ascribed the slow fluctuations (period of 15 s or greater and above) seen in human digital plethysmograms to the action of the thermoregulatory system. We have studied such a digital plethysmogram signal obtained from the small finger of a normal resting adult seated in a room maintained at 19° C. Apart from the faster components of period less than 15 s, which are probably due to the action of the blood pressure control system described above, the signal comprises only slow fluctuations thought to represent activity of the thermoregulatory system.

If the subject places one hand alternately in containers of hot (40° C) and cold (18° C) water for fixed intervals (say, 10 s, giving a 20 s period), the waveform of the digital blood flow signal is found to be quite different from the spontaneous signal. It comprises only a substantial component having the same period as the stimulus; "selective entrainment" seems to occur because in this case entrainment is possible while a similar magnitude stimulus of longer period, say, 2 min, is found to be ineffective.

We suggest that the spontaneous fluctuations in peripheral blood flow are due to adjustments in the cutaneous circulation by the thermoregulatory system. With the body core temperature at 37° C, thermal variations in the extremities are handled

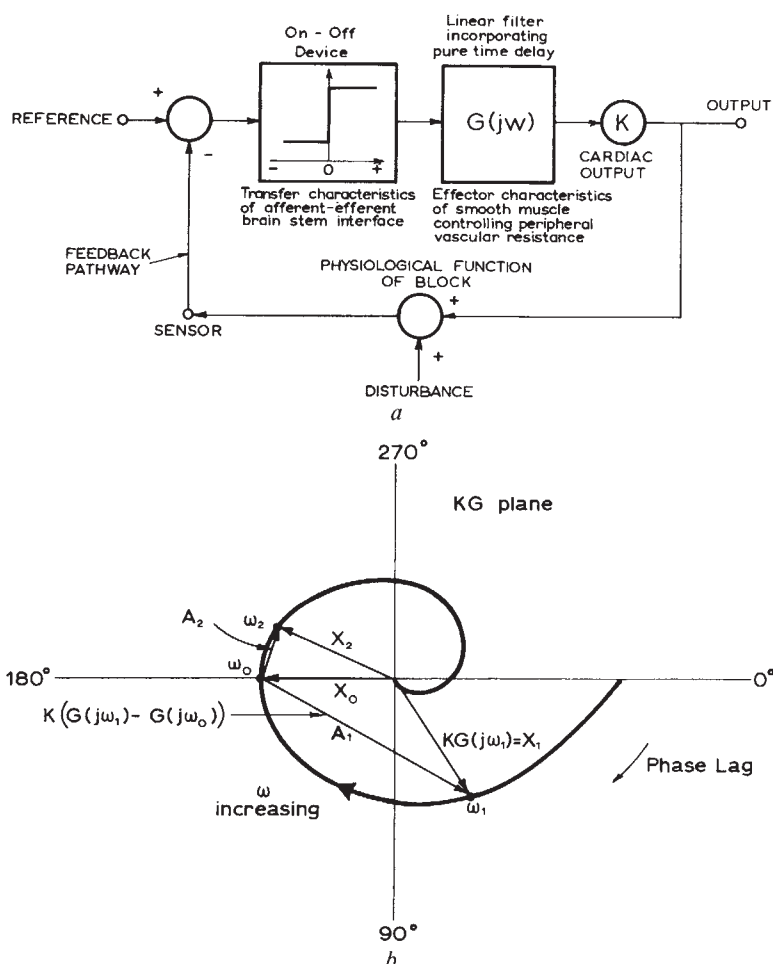


Fig. 2 a, Block diagram of a highly simplified version of the blood pressure control system incorporating only peripheral vascular resistance control. The on-off device represents the transfer characteristics of the afferent-efferent brain stem interface determined by Weidinger *et al.*⁴ b, Showing the minimum magnitude of the external disturbance at each frequency required in order to entrain the spontaneous oscillation (after Johnson⁵).

by the peripheral system. Selective entrainment occurs when a periodic thermal stimulus is applied, implying that the thermoregulatory system operates on a similar nonlinear basis to the blood pressure control system.

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- ¹ Dornhorst, A. C., Howard, P., and Leathart, G. L., *Circulation*, **6**, 553 (1952).
- ² Minorsky, N., *Nonlinear Oscillations* (Van Nostrand, London and New York, 1962).
- ³ Hyndman, B. W., thesis, Univ. London (1970).
- ⁴ Weidinger, H., Fedina, L., and Kehrel, H., *Pflügers Arch.*, **278**, 229 (1964).
- ⁵ Johnson, C. D., in *Nonlinear Automatic Control* (by Gibson, J. E.), 422 (McGraw-Hill, London and New York, 1963).
- ⁶ Fox, I. J., Crowley, jun., W. P., Grace, J. B., and Wood, E. H., *J. Appl. Physiol.*, **21**, 1553 (1966).
- ⁷ Nyquist, H., *Bell System Tech. J.*, **11**, 126 (1932).
- ⁸ Burton, A. C., *Amer. J. Physiol.*, **129**, 565 (1939).

Decreased Feeding after Injections of Amino-acids into the Hypothalamus

BUT for two exceptions^{1,2}, attempts to inhibit feeding behaviour by direct intrahypothalamic administration of glucose have been unsuccessful³⁻⁶. The effects of other nutrients have yet to be assessed. We have found that microinjections of balanced amino-acid solutions into dorsolateral perifornical hypothalamic tissue, where consummatory behaviours are obtained with electrical stimulation, inhibit feeding in rats.

Nine male albino rats with unilateral electrode-cannulae aimed for perifornical hypothalamic tissue were used. Each animal also had a bipolar electrode aimed at the corresponding area in the contralateral side of the hypothalamus for another study. They were caged individually and allowed free access to powdered lab chow and water except during short deprivation periods before feeding tests, and the cages received light for 12 h each day.

Initially the behaviour of each animal was observed during electrical stimulation (50 Hz, sine wave, 8–46 μ A) of the brain underlying the tip of the electrode-cannulae and for chemically induced feeding after cannulae-directed injections of 65 mM L-noradrenaline bitartrate dissolved in 90 mM NaCl in volumes of 1 μ l. Then the effects of intrahypothalamic injections of amino-acids were determined on three separate occasions. In the first two runs, the effects were compared with those of urea; in the third, they were compared with those of NaCl and D-glucose. Amino-acids and glucose were injected as 10% w/v solutions; urea and NaCl were injected as 4.2% w/v and 2.0% w/v solutions (approximately equiosmotic to the amino-acid solution). Each 10 g of the amino-acid mixture contained 0.33 g histidine HCl, 0.79 g lysine HCl, 0.29 g tryptophan, 0.72 g methionine, 0.72 g phenylalanine, 0.91 g leucine, 1.14 g isoleucine, 0.82 g valine, 0.72 g threonine, 2.60 g sodium glutamate, 0.31 g glutamine and 0.65 g glycine. L-Isomers were used. During all tests intrahypothalamic injections (1 μ l. each) were given 1 h 30 min and 5 min before the rats were given access to food. For the first run, food was removed 30 min before the start of the dark period and returned 1 h later; for the second and third runs, food was

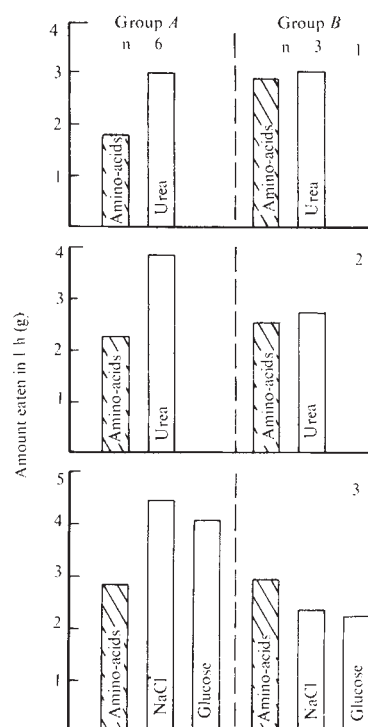


Fig. 1 Mean 1 h food intakes during three runs (see text).

removed 1 h after the start of the light period and returned 6 h later.

Mean 1 h food intakes for the three runs are presented in Fig. 1. In six subjects, amino-acids depressed feeding more than control injections during every test (group A) ($N_s=6$, $P_s<0.2$; randomization test for mixed pairs); in three they did not (group B). In no case did the injections produce visually distinguishable abnormalities in behaviour. The two groups also exhibited differential responses to injections of noradrenaline bitartrate and to electrical stimulation of tissue surrounding the tips of the cannulae. After noradrenaline injections, rats in group A ate 1.1 g (mean value) more than after saline injections; rats in group B ate only 0.4 g more ($n_1=6$, $n_2=3$, $V=1$, $P<0.5$; Mann Whitney test). Five rats in group A showed consummatory behaviour⁷ during electrical stimulation through the electrode-cannulae and also learned to self-stimulate for the current; the remaining subject in group A only self-stimulated. Animals in group B exhibited flight during electrical stimulation and none exhibited appetitive behaviour or self-stimulation. Histological studies indicated that cannulae tips of rats in group A were located in dorsolateral perifornical hypothalamus, where consummatory behaviour has been obtained with electrical stimulation, while those of group B were in the far lateral hypothalamus or in mid-lateral hypothalamus near the ventral surface of the brain.

These data complement recent reports that high systemic levels of amino-acids depress feeding more than comparable levels of the other major nutrients^{8,9} and suggest that circulating amino-acids depress feeding by a relatively direct action on hypothalamic neurones which are active during feeding behaviour. But the data do not necessarily imply the existence of a discrete sensory system which is responsive to blood amino-acids. Some amino-acids which are absorbed into the brain from the bloodstream¹⁰ slow neural firing rates nonspecifically¹¹⁻¹³. After the consumption of protein-rich meals, all neural activity, as well as that necessary for the facilitation of feeding, may be so affected. In any case, it is not yet known whether the observed effect reflects the operation of a physiological control mechanism.

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- ¹ Herberg, L. J., *Nature*, **187**, 245 (1960).
- ² Booth, D. A., *J. Comp. Physiol. Psychol.*, **65**, 13 (1968).
- ³ Epstein, A. N., *Amer. J. Physiol.*, **199**, 969 (1960).
- ⁴ Wagner, J. W., and DeGroot, J., *Amer. J. Physiol.*, **204**, 483 (1963).
- ⁵ Fisher, A. E. *Sci. Amer.* **210**, 60 (1964).
- ⁶ Grossman, S. P., *Fed. Proc.*, **27**, 1349 (1968).
- ⁷ Valenstein, E. S., Cox, V. C., and Kakolewski, J. W., *Psych. Rev.*, **77**, 16 (1970).
- ⁸ Adair, E. R., Miller, N. E., and Booth, D. A., *Commun. Behav. Biol.*, **2**, 25 (1968).
- ⁹ Panksepp, J., *Psychon. Monog. Suppl.* (in the press).
- ¹⁰ Henschen, A., and Söderberg, U., in *Structure and Function of Inhibitory Neuronal Mechanisms* (edit. by von Euler, C., Skoglund, S., and Söderberg, U.) (Pergamon, Oxford, 1968).
- ¹¹ Werman, R., Davidoff, R. A., and Aprison, M. H., *Nature*, **214**, 681 (1967).
- ¹² Curtis, D. R., Hösl, L., Johnston, J. H., and Johnston, G. A. R., *Exp. Brain Res.*, **5**, 235 (1968).
- ¹³ Curtis, D. R., Hösl, L., and Johnston, G. A. R., *Exp. Brain Res.*, **6**, 1 (1968).

Teratogenicity Studies of Methadone HCl in Rats and Rabbits

METHADONE maintenance was reported as a treatment for heroin addiction in 1965¹, and more than 10,000 patients are now maintained on the drug at more than 100 centres throughout the United States (unpublished Lilly Survey, 1970). Wallach *et al.*² observed that although women experienced abnormal menses during heroin addiction, regular menstruation and ovulation resumed with daily methadone treatment. In these studies several pregnancies were followed in which methadone maintenance was initiated before or during pregnancy with no apparent effect.

Guidelines for the clinical investigational use of methadone in maintenance programmes in the United States have been established only recently³. Because methadone was introduced as a narcotic analgesic several years before pre-clinical teratological studies were done, data about the effect of methadone in the pregnant animal are limited. In view of the lack of

animal reproduction studies involving the drug, and the limited documented clinical experience with methadone in pregnant patients, the recommended treatment for the pregnant addict is hospitalization and narcotic withdrawal.

Foetal anomalies and decreased litter size were observed in hamsters after females had received large subcutaneous doses of methadone on the eighth day of gestation⁴. We now present data for species more commonly used in teratological studies. Rats and rabbits were given doses ten to fifty times the average daily human maintenance dose.

Methadone HCl (*dl*-6-dimethylamino-4,4-diphenyl-3-heptanone hydrochloride) was administered to pregnant Harlan albino rats and Dutch belted rabbits as previously described⁵. The beginning of gestation (day 0) was determined in the rabbits by artificial insemination. The rats were mated, and the time of conception was noted by the presence of a copulatory plug. The rats and rabbits, in groups of twenty-five and fifteen respectively, were given daily oral doses of distilled water, or 20 or 40 mg/kg of methadone by gavage during organogenesis and early foetal development (gestation days 6–15 in the rat, 6–18 in the rabbit). Doses of 60 mg/kg, given in preliminary studies, induced unacceptable levels of toxicity (maternal death and/or foetal resorption). Foetuses were recovered before term by Caesarean section, and examined for external abnormalities and visceral or skeletal defects.

In rats, methadone produced distinct maternal toxicity similar to that of morphine^{6,7}. Hypertonia of skeletal muscle, which persisted up to 4 h after dosing, was often accompanied at the larger dose by catalepsy and respiratory depression; eight of the rats given methadone (three at the smaller dose) died of respiratory insufficiency. In rabbits, 40 mg/kg doses of methadone induced mild sedation during the early phases of the study. Two does on this regimen did not complete the test—one died on the twelfth day of gestation with signs of anorexia, weight loss and snuffles; the other aborted on the twenty-fifth day of gestation. The reproductive status of the animals that died was normal (necropsy evaluation); pregnancy was confirmed in seven of the eight rats and both rabbits.

In general, the treated rats ate and gained less than the control rats; in the rabbits, food intake was reduced in both the control and treated animals during the dosing period. These effects were not marked and were considered to be inconsequential in view of the negative teratological findings.

Pregnancy rates (Table 1) were depressed among animals on the 40 mg/kg regimen, but there was no sign of any impairment of reproduction. Normal litter sizes and numbers of corpora lutea indicated that the reduction in pregnancies was an isolated finding, unrelated to methadone treatment. Moreover, the fertility findings were not significantly variant from our cumulative control data (fertility in rats: range 70–100%, mean 90%; rabbits: range 40–100%, mean 80%). In treated rats resorption was slightly more frequent than in the controls; this was attributed to maternal toxicity.

Table 1 Uterine and Foetal Parameters

	Females pregnant	Mean No. foetuses $\pm$ s.e.	Resorptions Per total No. implantation sites	Females affected*	Mean foetal weight $\pm$ s.e. (g)
Rats					
Control	24/25	12.2 $\pm$ 0.6	15/308	10/24	3.87 $\pm$ 0.08
20 mg/kg methadone	25/25	13.0 $\pm$ 0.4	20/306	14/22	3.69 $\pm$ 0.05
40 mg/kg methadone	19/25	12.5 $\pm$ 0.7	26/214	11/15	3.66 $\pm$ 0.07†
Rabbits					
Control	15/15	5.1 $\pm$ 0.5	8/84	6/15	27.2 $\pm$ 1.2
20 mg/kg methadone	14/15	6.3 $\pm$ 0.7	9/97	6/14	25.5 $\pm$ 1.0
40 mg/kg methadone	11/15	5.7 $\pm$ 0.6	1/52	1/9	29.8 $\pm$ 0.9

* Based on pregnant females that survived.

† Not significant at 0.05 level, Dunnett's *t* test, Weil's number of independent sampling units^{8,9}.

Table 2 Foetal Abnormalities

	No. of foetuses examined		Incidence of abnormality			Skeletal examination	
	Rats	Rabbits	External examination Rudimentary tail	Internal hydrocephalus	Visceral examination Hydronephrosis	12 Ribs	
Control	293	76					
20 mg/kg methadone	286	88					
40 mg/kg methadone	188	51					
Cumulative control	9,016	1,620					
Rats*							
Control					24/107	1/186	
20 mg/kg methadone	1/286				12/103		
40 mg/kg methadone				1/66	6/66		
Cumulative control	1/9,016			8/3,739	478/3,739	1/5,277	
Rabbits							
	External examination	Visceral examination	Skeletal examination				Incomplete development
	Varus forepaws	Internal hydrocephalus	Hydronephrosis	13 Ribs	Sternebrae fused or extra	Sternal bars misaligned	cranial bones
Control		1/76		38/76	3/76	1/76	
20 mg/kg methadone	1/88 †	1/88 † ‡	1/88	50/88	5/88	1/88	1/88 † ‡
40 mg/kg methadone				26/51	6/51		
Cumulative control	2/1,620	2/1,620	1/1,620	444/1,620	17/1,620	11/1,620	22/1,620

* 1/3 of each litter was designated for visceral examination, 2/3 for skeletal examination. † Littermates. ‡ Same foetus.

Defects observed in the foetuses (Table 2) were representative of our control rat and rabbit populations. In the rats rudimentary tail, hydrocephalus and twelve ribs occurred as single, isolated defects; hydronephrosis was seen in foetuses of each treatment group, but most frequently in the control progeny. In the rabbits there was a low incidence of varus forepaws, hydrocephalus and hydronephrosis. Thirteen ribs, a common anatomical variation of our rabbits, and a few minor skeletal defects were observed as frequently in the control foetuses as in those of the methadone regimens.

Doses of 20 or 40 mg/kg of methadone administered orally to rats caused hypertonia of skeletal muscle and respiratory depression; some of the treated rats died of respiratory insufficiency. In rabbits, oral doses of 40 mg/kg induced mild sedation. Reproduction was not impaired in the rats or rabbits regardless of maternal condition. No drug-related defects were observed in the foetuses, and we concluded that methadone HCl in the doses which we used was not teratogenic in the rat or rabbit.

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Impairment of Recent Memory by Marihuana and THC in Rhesus Monkeys

SEVERAL studies with humans have indicated that marihuana and its active ingredient, delta-9-tetrahydro cannabinol (THC), have disruptive effects on short term memory¹⁻³. Of the few animal studies on THC, only that of Scheckel *et al.*⁴ addressed the question of memory impairment. The results of that study were attributed to lack of motivation. The study described here was designed to elucidate the effects of marihuana on short term memory in rhesus monkeys using an automated delayed matching-to-sample task. In our laboratory, monkeys have been trained to puff cigarettes for a water reward, and thus we are able to study the effects of marihuana smoke while avoiding the set problems associated with human subjects. Another group of monkeys, trained on a similar memory task, was administered oral THC. Significant memory impairment was established with both modes of administration.

In the first experiment, two adult male rhesus monkeys, trained to smoke cigarettes⁵, were placed on a two-task procedure for water reward. A smoking apparatus was attached to a 'Plexiglas' panel covering a 14 inch square opening on one side of each monkey's 36 inch cubic home cage. The smoking procedure has been described⁶; the only modification here was the absence of a dummy air tube, so that the monkeys were forced to puff smoke rather than having a choice of smoke versus air. A matching test was also attached to a 'Plexiglas' panel covering a 14 inch square opening on an adjacent side of each monkey's cage. The matching test has also been described elsewhere⁷. The combined smoking-matching procedure operated as follows: first, the monkey was required to make thirty puffs on the smoking machine (a fixed ratio 30 contingency was used throughout); this behaviour extinguished a light next to the puffing tube and signalled the start of the matching test. The monkey was then presented with a green or red sample light for 3 s on the matching panel. Touching a centre tube under the sample light turned off the light and, after a predetermined delay, two side

¹ Dole, V. P., and Nyswander, M., *J. Amer. Med. Assoc.*, **193**, 646 (1965).

² Wallach, R. C., Jerez, E., and Blinick, G., *Amer. J. Obstet. Gynec.*, **105**, 1226 (1969).

³ Edwards, C. C., *Federal Register*, **36**, 6075 (1971).

⁴ Geber, W. F., and Schramm, L. C., *Pharmacology*, **11**, 248 (1969).

⁵ Emmerson, J. L., Owen, N. V., Koenig, G. R., Markham, J. K., and Anderson, R. C., *Toxicol. Appl. Pharmacol.*, **19**, 471 (1971).

⁶ Scott, C. C., and Chen, K. K., *J. Pharmacol. Exp. Therap.*, **87**, 63 (1946).

⁷ Chen, K. K., *Ann. NY Acad. Sci.*, **51**, 83 (1948).

⁸ Dunnett, C. W., *Biometrics*, **20**, 482 (1964).

⁹ Weil, C. S., *Food Cosmet. Toxicol.*, **8**, 177 (1970).

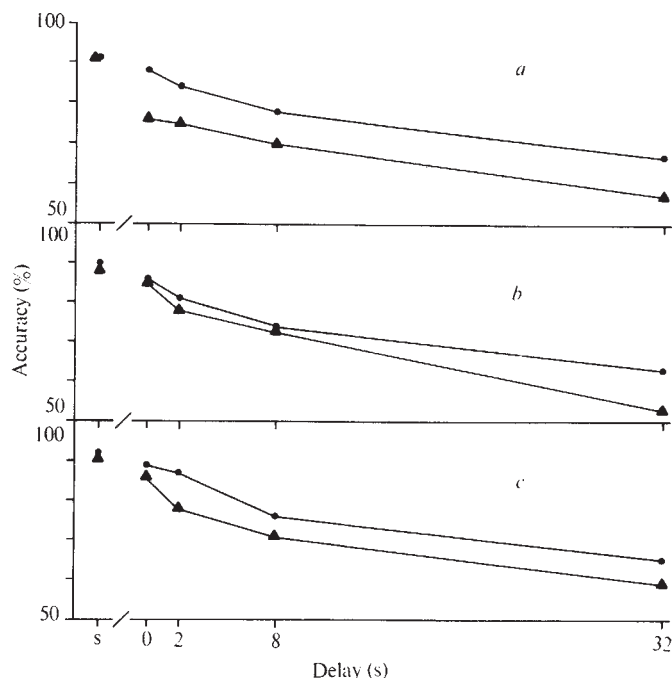


Fig. 1 Effect of THC on delayed oddity performance (s = simultaneous). ●, Control; ▲: a, 2.0 mg/kg THC; b, 1.0 mg/kg THC; c, 0.5 mg/kg THC.

lights appeared, one green and one red. Touching the solenoid of the light which matched in colour the previous sample light resulted in a 2 ml. water reward. If an error was made, or if no response was made after 8 s, the side lights were turned off and the light of the smoking machine appeared, signalling the start of the smoking test again. The monkeys were tested on a total of three delays in the matching test: 0 s, 5 s and 30 s; however, only two delays were used during the same session (either 0 and 30 s, 5 and 30 s, or 0 and 5 s). The delays were programmed randomly and the delay combinations were presented in a balanced order to control for possible sequence effects. Testing was programmed automatically by Digibit logic circuits as well as by punched tape devices, all of which were located in a separate control room.

The monkeys were tested in the two-task procedure 7 days a week and received all their water during a daily 4 h testing session (starting at 1030 h). On all days the monkeys were given access to thirty cigarettes. On control days the subjects received tobacco cigarettes (Raleigh plains). On drug days (Tuesdays and Fridays) thirty marihuana cigarettes, rolled in the laboratory, were used, with tobacco plugs at either end. Each cigarette contained approximately 0.5 g of marihuana, obtained from *Cannabis sativa*, Mexican female, 1969, which was supplied by DHEW, Public Health Service, NIMH (analysis: THC, 1.5%; cannabidiol, 0.12%). The effect of marihuana on each monkey's performance on each delay was tested an average of six times. The total number of smoking puffs per session, the total number of matching trials taken, as well as the accuracy on each delay, were recorded and analysed. Drug days and control days were compared, the day immediately preceding the drug day being considered as the control day. Chi-square tests were used to compare statistically the differences.

The results (data for two monkeys combined) are presented in Table 1. Accuracy was lowered but not significantly impaired by marihuana at 0 s ($P > 0.2$) and 5 s (just missed significance with $P = 0.06$). On the 30 s delay, however, accuracy was significantly impaired ($P < 0.01$) by marihuana. The rate of puffing smoke as well as the rate of responding on the matching test were both significantly decreased ($P < 0.05$ in both cases). (There were no significant differences between

Table 1 Effect of Marihuana Smoking on Delayed Matching Performance

Accuracy %	Marihuana	Control
0 s	99	100
5 s	89	94
30 s	68	74
Total puffs	4,614	5,795
Total matching trials	150	198

the two monkeys on any performance measure in any drug or control condition.)

In the second experiment, four adult rhesus monkeys (different monkeys from those used in the first experiment) were used, all with extensive previous experience on the memory task. The task was similar to the matching test used in the first experiment, except that instead of matching the colour of one of the side lights to the sample light, the monkey had to choose the odd colour⁸. The oddity test was attached to a 'Plexiglas' panel covering a 14 inch square opening on one side of each monkey's 36 inch cubic home cage. On each trial one of five delay conditions was used: simultaneous, 0, 2, 8 and 32 s. They were presented in randomized order such that each animal received 960 trials (192 for each delay) during an 8 h session (starting at 1000 h). For the simultaneous presentation a red or a green light (the sample stimulus) was presented on the central display unit at which time the animal was required to touch a centre tube (under the sample light) within 3 s. A response to the sample stimulus immediately resulted in presentation of one red light and one green light (the comparison stimuli) on each of the two side units, respectively, for up to 8 s; the sample stimulus remained on with the comparison stimuli. The monkey then had to non-match (oddy) one of the side comparison stimuli with the colour of the sample stimulus by touching the correct tube in order to receive a water reward (1 ml.). The procedure for the real delay conditions was the same, except that the sample stimulus appeared alone and terminated some time before the comparison stimuli appeared. The monkeys were tested 7 days a week and received all their water in the test conditions.

The THC (supplied by NIMH) was diluted in 10 ml. of apple juice and administered orally. On control days 10 ml. of apple juice alone was administered. The monkeys drank the juice from a small plastic bottle through the front of their cages. The juice or juice-drug mixture was administered 15 min before testing. Drug days were Tuesdays and Fridays; six drug administrations were given, one at 0.5 mg/kg, three at 1 mg/kg and two at 2 mg/kg. The accuracy (% correct) and rate (% responses made out of possible total) data recorded on punched tapes were sorted and summed for drug and control days according to delay conditions. Again, the day before the drug day was considered the matched control day. Chi-square tests were used for data analysis.

The accuracy results (data for four monkeys combined) are presented in Fig. 1. Accuracy was significantly impaired by 0.5 mg/kg of THC on the 2 s and 32 s delays ($P < 0.05$ in both cases); by 1.0 mg/kg on the 32 s delay ($P < 0.05$); and by 2.0 mg/kg on the 0, 2, 8 and 32 s delays ($P < 0.05$, 0.05, 0.05 and 0.01, respectively). Accuracy was never impaired when sample and comparison stimuli were simultaneous. The rate of responding was significantly decreased by all doses of the drug in all testing conditions ($P < 0.01$ in all cases). The percentage of responses, taken out of the total offered, was approximately 60% on control days, 48% for 0.5 mg/kg THC, 39% for 1.0 mg/kg THC and 27% for 2.0 mg/kg THC.

In both the marihuana and THC studies, both the rate of responding and the accuracy were affected. The rate effects possibly confirm the conclusion⁴ that THC induces a "loss of ability or motivations to perform complex tasks". But our results also strongly suggest that marihuana and THC have a definite effect on short term retention processes *per se*. In

the smoking-matching test, marihuana lowered accuracy only when a 30 s delay was used. In the oddity test, THC impaired accuracy only in delay conditions and never in the simultaneous condition. This type of impairment is different from that shown with amphetamine, scopolamine, chlorpromazine, pentobarbital, LSD^{7,9-11} or mescaline (unpublished data) on a similar task. By comparison with these other drugs, the specificity of THC is indeed remarkable.

Unlike Scheckel *et al.*⁴ we obtained no evidence of long term effects. Performance on days immediately following drug administrations was always similar to that on days preceding them. Scheckel *et al.*⁴ used squirrel monkeys and we used rhesus monkeys; this species difference may explain the variant results. We also failed to find objective criteria which would warrant the assumption of hallucinations⁴ in our monkeys; their behaviour may have changed quantitatively, but not qualitatively. Scheckel *et al.*⁴ also reported a biphasic effect of THC on response rate, with depression occurring at low doses and stimulation at high doses (16, 32 and 64 mg/kg). As more than half of their monkeys died after being administered these high doses, we were unwilling to attempt a confirmation of possible excitation effects.

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- ¹ Tinklenberg, J. R., Melges, F. T., Hollister, L. E., and Gillespie, H. K., *Nature*, **226**, 1171 (1970).
- ² Melges, F. T., Tinklenberg, J. R., Hollister, L. E., and Gillespie, H. K., *Science*, **168**, 1118 (1970).
- ³ Weil, A. T., and Zinberg, N. E., *Nature*, **222**, 434 (1969).
- ⁴ Scheckel, C. L., Boff, E., Dahlen, P., and Smart, T., *Science*, **160**, 1467 (1968).
- ⁵ Pybus, R., Goldfarb, T., and Jarvik, M. E., *J. Exp. Anal. Behav.*, **12**, 88 (1969).
- ⁶ Glick, S. D., Canfield, J. L., and Jarvik, M. E., *Psychol. Rep.*, **26**, 707 (1970).
- ⁷ Glick, S. D., and Jarvik, M. E., *J. Pharmacol. Exp. Ther.*, **169**, 1 (1969).
- ⁸ Glick, S. D., Nakamura, R. K., and Jarvik, M. E., *Commun. Behav. Biol.*, **5**, 165 (1970).
- ⁹ Robustelli, F., Glick, S. D., Goldfarb, T. L., Geller, A., and Jarvik, M. E., *Commun. Behav. Biol.*, **3**, 101 (1969).
- ¹⁰ Glick, S. D., Goldfarb, T. L., Robustelli, F., Geller, A., and Jarvik, M. E., *Psychopharmacologia*, **15**, 125 (1969).
- ¹¹ Glick, S. D., and Jarvik, M. E., *Fed. Proc.*, Atlantic City, New Jersey (1970).

Oddity and Specific Searching Image more Important than Conspicuousness in Prey Selection

In an earlier report¹ I presented results from a pilot experiment which suggested that conspicuousness of prey was the most important determinant in prey selection. I now have results from 1,600 laboratory experiments performed with two species of hawks in rigorous experimental conditions, which suggest that oddity and the specific searching image are more important. Many observations and experimental results fit well into the generalization that predators have a tendency to select odd prey, animals that differ in some way from most prey^{2,3}, although unequivocal evidence is lacking. Predators also show a tendency to continue to select a given type of prey, even though other types may be as readily, or even more easily

available, a phenomenon that L. Tinbergen⁴ has termed the "specific searching image". My experiments are a simultaneous test of three factors which influence prey selection: conspicuousness, oddity, and the specific searching image.

Six tamed American kestrels (*Falco sparverius*) and two broad-winged hawks (*Buteo platypterus*) were allowed to prey on white laboratory mice, some of which had been coloured grey with food dye. In each experiment, nine mice of one colour and one of the other were presented on a substrate which was painted to match one or the other colours of mice. Each of the two colour combinations of mice was replicated fifty times on each of the two colours of substrate, giving a total of 200 experiments for each bird (Table 1). The sequence in which given mouse and substrate combinations (A, B, C, D) were presented to the hawk was randomized. The hawk was allowed to select only one mouse per experiment; the expected number of captures of each colour of mouse, assuming no (or random) selection is given in Table 1. The mice were presented individually on pedestals about 10 cm square and 15 cm high which were arranged about 10 cm apart on an arc of a circle of approximately 2 m radius. The hawk was placed on a perch at the centre of the circle. Laboratory mice rarely leave such a pedestal and the distribution of mice can thus be controlled. The position of the odd mouse in each experiment was randomized.

All eight hawks showed a statistically significant preference for odd mice, but only of one colour; four for white, odd mice and four for grey, odd mice (Table 2). In two cases the preference for white was sufficiently strong that no grey (and odd) mice were taken from a grey substrate. Three birds took significantly more odd mice of the preferred colour when these differed in colour from the substrate than when the mice matched the substrate. Thus mice of a particular colour were preferred, but only when they were odd, and the conspicuousness of the mouse had relatively little influence on the results.

My results thus suggest that the specific searching image may be the major determinant in prey selection in hawks although oddity is very nearly as important. I am conducting

Table 1 Experimental Design

Series	No. of experiments	Substrate	Mice presented in each experiment		Expected total catch*	
			Grey	White	Grey	White
A	50	Grey	9	1	45	5
B	50	Grey	1	9	5	45
C	50	White	9	1	45	5
D	50	White	1	9	5	45

* If there is no, or random, selection.

Table 2 Number of Odd Mice Taken

Hawk	Sex	Experiment series			
		A	B	C	D
Lucy	♀	4	16*	2	16*
Gimpy	♀	25*	6	21*	6
Bilbo	♂	31*	0*	18*	4
Gandalf	♂	48*	0*	31*	1
Frodo	♂	10*	4	15*	9
Arwen	♀	4	16*	2	21*
Baby	♂	5	14*	3	20*
Ignatz	♂	6	22*	2	37*

* Departs significantly from the expected value, chisquare test, $P < 0.05$. Baby and Ignatz are *B. platypterus*; all other individuals are *F. sparverius*.

further experiments in an attempt to separate, and assess, the relative importance of these two factors.

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¹ Mueller, H. C., *Nature*, **217**, 92 (1968).

² Salt, G. W., *Ecol. Monogr.*, **37**, 113 (1967).

³ Pielowski, Z., *Ekologia Polska (A)* Tom IX No. 11 (1961).

⁴ Tinbergen, L., *Arch. Neer. Zool.*, **13**, 265 (1960).

CO₂-dependent Oxygen Evolution by Envelope-free Chloroplasts

THE chloroplasts isolated by Hill¹ in the 1930s were capable of rapid rates of oxygen evolution when provided with an artificial electron acceptor (oxidant) but did not retain the ability to assimilate CO₂ at rates detectable by the methods then available. Nearly 20 yr later the ability of isolated chloroplasts to fix CO₂ was demonstrated by Arnon, Allen and Whatley² but even then the rates were only 3–6% of those supported by the intact plant. By 1966, when the rates of fixation by chloroplasts isolated in sugar media first matched those of intact leaves^{3,4}, it had been established^{5,6} that high rates of carbon assimilation were only achieved by chloroplasts with intact envelopes. It is unlikely that all the earlier difficulties were caused by the unintentional isolation of damaged chloroplasts but, except for some early work by Arnon⁷ (in which chloroplasts were supplemented with cytoplasmic malic enzyme), it has certainly not been possible to show CO₂-dependent oxygen evolution by envelope-free chloroplasts⁸ and rapid rates (comparable with the Hill reaction) have not been previously reported. Such high rates have now been observed in a reconstituted system containing ATP, ferredoxin, NADP (nicotinamide adenine dinucleotide phosphate), R5P (ribose-5-phosphate), CE (soluble components released from osmotically shocked chloroplasts) and envelope-free chloroplasts.

Fig. 1 shows that oxygen was evolved immediately after illumination of a mixture from which PGA (3-phosphoglycerate) and R5P were initially omitted. This evolution is attributable to the reduction of NADP and it ceased (or slowed, depending on the activity and content of the chloroplast extract) according to the stoichiometry of equation (1).



At this point evolution can be restarted by the addition of PGA^{9,10} or by the addition of R5P (if CO₂/bicarbonate is present), and it can be assumed that both of these compounds lead (by the appropriate reactions of the Benson–Calvin cycle) to the formation of DPGA (1,3-diphosphoglycerate) which then regenerates NADP in the oxidized form according to equation (2).



The response to the addition of PGA is very rapid (Fig. 1A) and the maximum rate is attained within seconds. The response to R5P depends to some extent on the activity of the preparation but it is always detectably slower (Fig. 1B) than the response

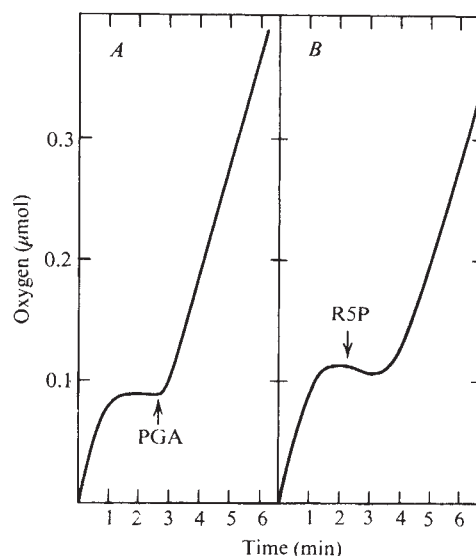


Fig. 1 Oxygen evolution by envelope-free chloroplasts initiated (A) by adding PGA or (B) by adding R5P. Only reaction mixture B contained added CO₂/bicarbonate. Intact chloroplasts were prepared in sorbitol medium⁶ from 80 g pre-illuminated spinach leaf and freed of their envelopes by resuspending in 10 ml. assay medium (AM) at 1/25 full strength. NaCl was then added to a final concentration of 0.1 M. After a second centrifugation the envelope-free chloroplasts⁶ were resuspended in AM and the supernatant retained and designated CE^{9,10}. The chloroplasts from which the fraction CE was derived contained a total of 4 mg chlorophyll. The protein content of CE was 12 mg/ml. Each 2 ml. reaction mixture contained CE (0.9 ml.) envelope-free chloroplasts (100 μg chlorophyll) double strength AM (1 ml.) ferredoxin (3 nmol) and NADP (0.25 μmol). AM contained HEPES¹⁷ (50 mM at pH 7.6), sorbitol (0.33 M), EDTA (2.0 mM) MgCl₂ (1.0 mM), MnCl₂ (1.0 mM). In A, the reaction mixture also contained 0.4 μmol of ATP and 3 μmol of PGA were added as indicated. In B, 4 μmol of ATP and 200 μmol of NaHCO₃ were added at the outset and 3 μmol of R5P as indicated. Oxygen evolution was measured in twin oxygen electrodes at 20° C in saturating red light⁹.

to PGA and in some experiments the rate was not maximal for 2–3 min.

Oxygen evolution could be initiated by PGA in the absence of added CO₂ (Fig. 1A) whereas with R5P (Fig. 1B) CO₂/bicarbonate was also needed. This requirement for bicarbonate is illustrated in Fig. 2. In this experiment, as in Fig. 1, the MgCl₂ content of the reaction mixtures was the same as that used in earlier experiments with intact chloroplasts (for example, ref. 6). At this concentration of MgCl₂, however, it is necessary to add CO₂/bicarbonate in ten-fold excess of that needed to saturate intact chloroplasts. The addition (Fig. 2) of the necessarily large volume (0.2 ml.) of bicarbonate solution produces a dip in the oxygen electrode trace (owing to the problem of matching the oxygen concentration of the reaction mixture with that of the added bicarbonate solution). This causes some difficulty in interpreting the kinetics of response but many experiments of this sort have led us to the conclusion that there is again a finite lag between the addition of bicarbonate and the subsequent attainment of the maximum rate (compare the addition of R5P in Fig. 1). If the concentration of MgCl₂ is raised by 4 mM there may be measurable oxygen evolution in the absence of added bicarbonate and saturation is reached at bicarbonate concentration closer to 10 mM (compare refs 11–14).

The CO₂-dependent oxygen evolution was observed only in the presence of added ATP. The response to added ATP was immediate (unlike that to R5P or CO₂/bicarbonate) but the immediacy of this reaction may be partly related to the further conversion of small quantities of PGA (newly formed at the expense of endogenous ATP) rather than to an excep-

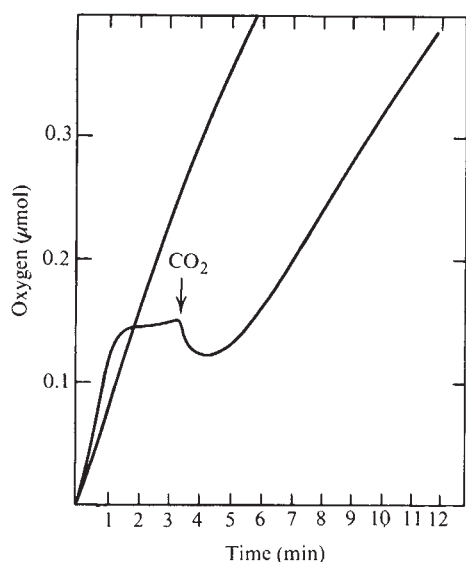


Fig. 2 CO_2 -dependent oxygen by envelope-free chloroplasts. As for Fig. 1 except that both reaction mixtures contained ATP (5 μmol) and R5P (3 μmol) from the outset. CO_2 /bicarbonate (200 μmol) was added before illumination or as indicated.

tionally rapid formation of triose phosphate from R5P. In most experiments there was no CO_2 -dependent oxygen evolution unless ATP was added in excess of that required to saturate PGA-dependent oxygen evolution. Following the addition of ATP to reaction mixtures containing limiting quantities of chlorophyll (20 μg) which have been pre-illuminated (2 min) we have observed rates of evolution as high as 175 $\mu\text{mol}/\text{mg}$ chlorophyll/h. When there has been no possibility of prior accumulation of PGA from R5P and CO_2 (for example, when R5P and NADPH_2 have been added simultaneously at the onset of illumination) the maximum rates were nearer 80 $\mu\text{mol}/\text{mg}/\text{h}$ but under these conditions it was often impossible to obtain much faster rates with added PGA as substrate (Fig. 1) and it is probable that accumulation of PGA is not the only factor which contributes to the highest rates observed.

Our results show that it is possible to reconstitute a system in which completely envelope-free chloroplasts will support a carbon dioxide-dependent oxygen evolution and that this system requires added ATP and R5P. For maximal rates it is necessary to increase either the bicarbonate concentration or the Mg^{2+} concentration substantially beyond the respective optima for intact chloroplasts. Indirectly, our observations confirm and extends those of Whatley *et al.*¹⁵ who first demonstrated CO_2 fixation by broken chloroplasts fortified with CE. Our approach is essentially similar but in the light of recent knowledge we have also been able to vary the nature and concentration of additives in such a way that we were able to obtain better rates (assuming an incubation period of 1 h, the figure given by Whatley *et al.* for CO_2 fixed with R5P corresponds to a rate of 8 $\mu\text{mol}/\text{mg}$ chlorophyll/h).

Without the constraints imposed by physical compartmentation and biochemical regulation it is improbable that it will ever be possible to reconstitute, in one simple suspension of illuminated envelope-free chloroplasts, a system capable of operating the entire Benson-Calvin cycle, as a cycle. That long sequences of the complete system can be made operable, however, indicates that these are not (in themselves) dependent on an intact structure, except to the extent that structure defines the environment within the chloroplast in which the reactions normally occur. Further investigation of the reconstituted system could clearly lead to a better understanding of these environmental requirements including those which govern the affinity of the principal carboxylating enzyme for its substrate¹⁶.

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- ¹ Hill, R., *Nature*, **139**, 881 (1937).
- ² Arnon, D. I., Allen, M. B., and Whatley, F. R., *Nature*, **174**, 394 (1954).
- ³ Bucke, C., Walker, D. A., and Baldry, C. W., *Biochem. J.*, **101**, 636 (1966).
- ⁴ Jensen, R. G., and Bassham, J. A., *Proc. US Nat. Acad. Sci.*, **56**, 1095 (1966).
- ⁵ Walker, D. A., *Plant Physiol.*, **40**, 1157 (1965).
- ⁶ Walker, D. A., in *Biochemistry of Chloroplasts* (edit. by Goodwin, T. W.), Proc. NATO Adv. Study Inst., Aberystwyth, 1965, 2, 53 (Academic, New York, 1966).
- ⁷ Arnon, D. I., *Nature*, **167**, 1008 (1951).
- ⁸ Walker, D. A., and Crofts, A. R., *Ann. Rev. Biochem.*, **36**, 389 (1970).
- ⁹ Stokes, D. M., and Walker, D. A., *Plant Physiol.* (in the press).
- ¹⁰ Stokes, D. M., and Walker, D. A., *Photosynthesis and Photorespiration* (edit. by Hatch, M. D., Osmond, C. B., and Slatyer, R. O.) (Wiley Interscience, New York, 1971).
- ¹¹ Sugiyama, T., Matsumoto, C., and Akazawa, T., *Biochem. Biophys. Res. Commun.*, **30**, 118 (1968).
- ¹² Sugiyama, T., Nakayama, N., and Akazawa, T., *Arch. Biochem. Biophys.*, **126**, 737 (1968).
- ¹³ Bassham, J. A., *Science*, **172**, 526 (1971).
- ¹⁴ Preiss, J., and Kosuge, T., *Ann. Rev. Plant Physiol.*, **21**, 433 (1970).
- ¹⁵ Whatley, F. R., Allen, M. B., Rosenberg, L. L., Capindale, J. B., and Arnon, D. I., *Biochim. Biophys. Acta*, **20**, 462 (1956).
- ¹⁶ Walker, D. A., *Nature*, **226**, 1204 (1970).
- ¹⁷ Good, N. E., Winget, G. D., Winter, W., Connelly, T. N., Isawa, S., and Singh, R. M. M., *Biochemistry*, **5**, 467 (1966).

Elimination of the Response to Gravity of Lettuce Seedlings

WHILE investigating the effects of certain lactones on the germination of lettuce seeds I have found that 3-4' chlorophenyl 3-methoxy phthalide (I) modifies the growth of seedlings. Jones *et al.* found that this compound and other derivatives of 2-benzoylbenzoic acids—either as the free acid or as lactones—affected the growth, geotropic and phototropic behaviour of seedlings.

Radicles and hypocotyls of most seedlings are strongly orthogeotropic^{2,3}. Synthetic growth regulators with physiological action like that of IAA (β indol-3-yl acetic acid) affect root and shoot growth, sometimes making these organs grow in an unexpected direction. Antitropistic effects can also be induced by morphactins (derivatives of 9-hydroxy-9-carboxylic acid fluorene)⁴, N- α -naphthyl phthalamic acid (NAP)⁵, and tri-iodo benzoic acid (TIBA)⁶.

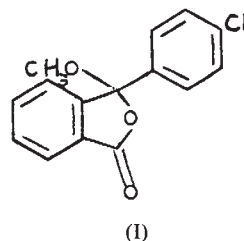


Table 1 Orientation of Radicles and Hypocotyls of Lettuce Seedlings Treated with 3-4' Chlorophenyl 3-Methoxy Phthalide (I) (Effective Concentration Saturated Aqueous Solution)

	Degrees from horizontal	
	(negative upwards) Radicle	(positive downwards) Hypocotyl
Control	+74.06 ± 4.79	-94.94 ± 1.52
Treated	-20.50 ± 5.18	-0.44 ± 2.57

Compound (I) eliminates the geotropic response of the seedling, and the direction of growth is then determined by the initial orientation of the embryonic axis. Lettuce, cultivar Grand Rapids, or mustard seedlings were grown either on seed test paper which was loaded with (I), or from seed which had been soaked in a saturated aqueous solution of (I) at 4° C for 48 h. (Seed test paper 90 mm by 0.4 mm thick can be loaded with a given weight of physiologically active material in an organic solvent. After this has evaporated water is added to provide an aqueous milieu equivalent to a given molarity. Although this may seem to be greater than saturation concentrations the fat content of the seed ensures that the active material is present at an endogenous level corresponding to the stated molar concentrations. Control substratum was treated with the solvents used.)

Seeds were placed on the paper so that the radicle pointed in the desired direction. Because small seeds adhere readily to seed test paper, I usually positioned seeds on the paper lining the base of the Petri dish and then set the paper vertically. Using another method, I folded seed test paper (90 mm × 0.4 mm thick) to give a centre upright fold secured by two paper clips. Seeds were inserted into the apex of the fold and held in any desired position. These assemblies were placed in plastic trays (15 cm × 20 cm) containing about 50 ml. of deionized water. The seeds germinated and the seedlings developed at 20° C in darkness. Phototropic curvature was induced in 72 h dark grown seedlings derived from vertically oriented embryos, radicle down, by irradiating unilaterally with a 40 W tungsten bulb, run at mains voltage (240) 75 cm from the seedlings. In all experiments on phototropism at least nine, and usually sixteen, individuals were measured. Each of these experiments was performed at least twice. Observations on

the statoliths of the hypocotyl of mustard were made on freshly cut sections stained in aqueous iodine.

Elimination of geotropism by compound (I) was demonstrated by placing sixteen lettuce seeds at appropriate compass points on vertically positioned Petri dishes, with radicles facing outwards. The paper was loaded with (I) to a final concentration equivalent to 10⁻⁴ M. Fig. 1 shows these plates after 72 h at 20° C. Clearly (I) affects seedlings so that the direction of growth of the axis is determined by the original orientation. Fig. 1 also shows that the plumular hook does not form in treated material, and is only manifest in control material when the axis is off centre or inverted—note the appearance of the lowermost seedling in the control dish.

A better measure of the response of the seedlings to (I) was obtained by placing seeds horizontally and measuring the angle between the radicle or hypocotyl and the horizontal axis. Above horizontal attitudes are prefixed by a negative sign to agree with conventional nomenclature of orthogeotropic behaviour. Table 1 lists one set of results.

To compare growth by radicles and hypocotyls of treated seed, seeds were distributed on seed test paper as described, the paper being loaded with appropriate mixtures.

Table 2 shows that the growth of radicles of treated seeds was reduced by about 20% while the growth of hypocotyls was reduced by about 40%. As IAA is involved in tropic responses and gibberellic acid (GA) in extension growth, the effect of coapplication of each of these with (I) was examined. Table 2 shows that 10⁻⁴ M GA overcomes the slight inhibition of growth caused by (I) without any effect on the elimination of the geotropic response. IAA severely decreases the extension of the radicle, as expected, but there is evidence that at 10⁻⁵ M it can re-establish normal geotropic behaviour at the expense of growth in some radicles.

Phototropic behaviour is affected less by (I) (Table 3). The slower response of the treated material may be a result of the reduction in extension growth that (I) causes, and it is possible that the compound was used in a concentration less than that which would have eliminated phototropism. The results of Jones *et al.*¹, while not strictly quantitative, indicate that these compounds affect phototropism less than geotropism.

A possible point of action of (I) on the geotropic response is the statolith, which many people think is a central feature in the perception of gravity^{2,3}. If statolith starch were lost,

Table 2 Growth, Geotropic Response and Development of Plumular Hook in the Young Axis of Lettuce Seedlings Treated with (I), GA, and IAA Singly and in Combination

	Radicle			Hypocotyl			Plumular hook
	Geotropic response		Length (mm)	Geotropic response		Length (mm)	
	+ ve	none		– ve	none		
Control	30	0	20.10 ± 0.916	30	0	5.00 ± 0.352	+
10 ^{–4} M (I)	0	31	16.13 ± 0.897	0	31	2.87 ± 0.225	–
10 ^{–4} M (I)+ 10 ^{–5} M GA	0	32	16.03 ± 0.823	0	32	4.09 ± 0.341	–
10 ^{–4} M (I)+ 10 ^{–4} M GA	0	31	20.19 ± 0.500	0	31	4.97 ± 0.359	–
10 ^{–4} M (I)+ 10 ^{–3} M GA	0	32	18.09 ± 0.792	0	32	4.53 ± 0.327	–
10 ^{–4} M (I)+ 10 ^{–7} M IAA	0	32	9.41 ± 0.609	0	32	2.13 ± 0.257	–
10 ^{–4} M (I)+ 10 ^{–6} M IAA	2	30	5.91 ± 0.500	†	†	1.66 ± 0.122	–
10 ^{–4} M (I)+ 10 ^{–5} M IAA	15	17	2.37 ± 0.396	†	†	†	†

† Not measurable.

Table 3 Phototropic Curvature of Lettuce Seedling Hypocotyls grown in the Presence of a Saturated Solution of (I)

Length of exposure to light (h)	Degrees curvature from vertical	
	Treated	Control
7.5	5.8 ± 2.46	29.3 ± 1.53
24.0	41.1 ± 7.27	51.5 ± 2.85

then the axis could not perceive the stimulus⁷, or if the starch grains could not move under gravity than it might be expected that there would be no curvature when the axis is placed off vertical. Fig. 2 shows that the starch grains in the endodermis of mustard hypocotyl, often considered to be statoliths, are present in treated material, and move in response to gravity. They can be seen congregated along the basal wall of the cell in vertically grown hypocotyls, but are less distinctly oriented in seedlings which were growing horizontally.

According to the Cholodny-Went hypothesis, curvatures

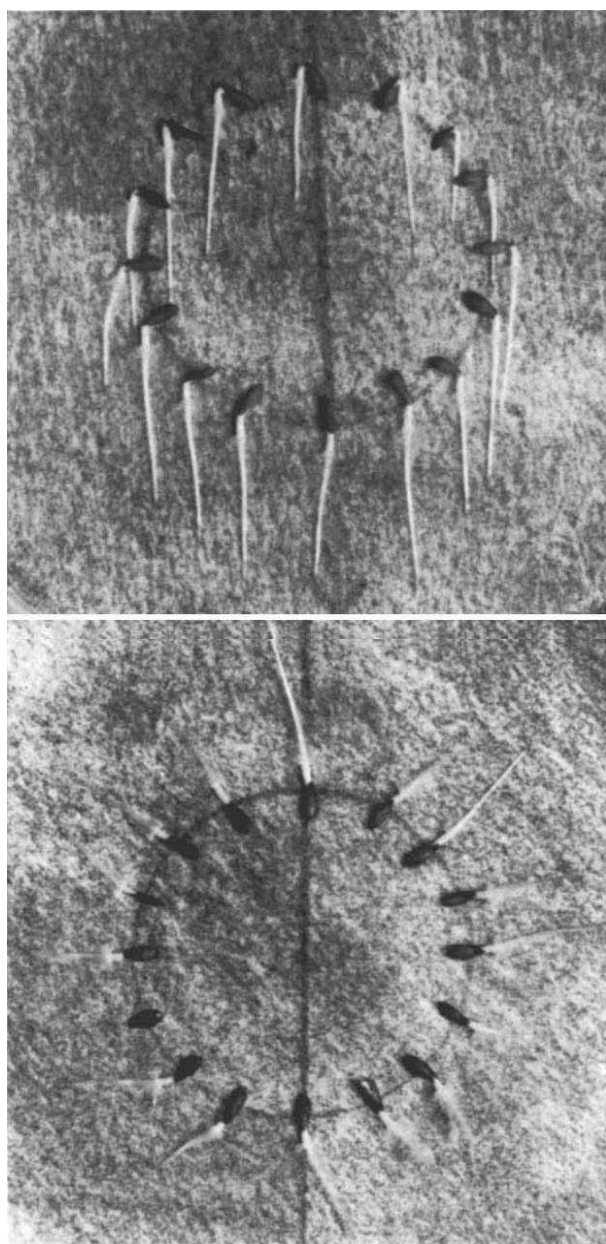


Fig. 1 Seedlings from lettuce seed in which embryonic axis is positioned with respect to given compass points. Upper—control. Lower—treated with 3-4' chlorophenyl 3-methoxy phthalide.

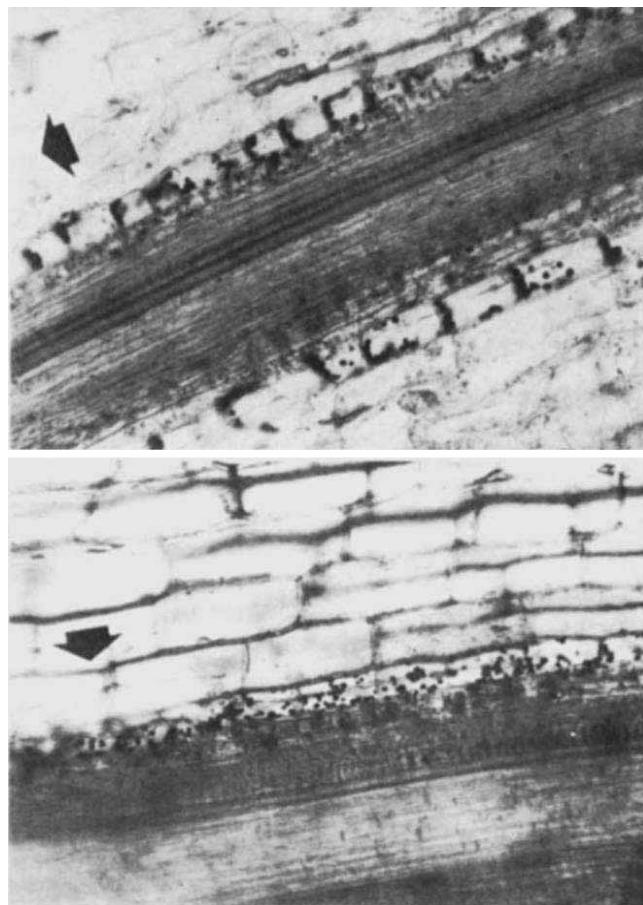


Fig. 2 Statolith orientation in hypocotyl endodermis of mustard. Arrows indicate direction of gravity.

result from the misorientation of an organ in the gravitational field, or from unilateral illumination, because the unequal distribution of auxin across the width of the organ gives unequal bilateral growth in the region of cell elongation^{8,9}. At least for lettuce seedlings (I) can prevent curvature in a gravitational field but not in the case of unidirectional illumination. If the Cholodny-Went hypothesis is accepted, and the phototropic curvatures of seedlings treated with (I) result from unequal bilateral distribution of auxin, then it might be argued that the establishment of this bilateral asymmetry in auxin concentration is *per se* not affected by (I). The effect of (I) on geotropic sensitivity of seedlings must be sought elsewhere.

Audus suggests that geotropism is a catenary process with several interconnected links, each requiring to be completed before the phenomenon can be expressed properly². The first link relates to the site of perception, and if this is the statolith then (I), which has no observable effect on the starch grains of the endodermal cell of the hypocotyl of mustard, is unlikely to act directly on the statolith. I do not know which link in Audus's chain of events is affected by (I), but this compound clearly provides a useful tool for the study of this phenomenon.

The reversal by GA of the slight growth inhibition caused by (I) in the roots and hypocotyls suggests that (I) competes with endogenous GA for the site of action of this hormone. This is in agreement with the idea that candidate antigibberellins should occur among unsaturated lactones¹⁰, which include coumarin¹¹, ramulosin¹² and patulin¹³. It is well known that these and other unsaturated lactones are potent physiologically active compounds in many classes of organism¹⁴. So far,

however, such lactones seem to be restricted in their anti-gibberellin activity, being most noticeably active in the lettuce seed germination assay. Inhibition of GA-induced elongation of organs is less common. Perhaps the inhibition of elongation by (I) is sufficient to mask the growth differential across the elongating organ in geotropism but not in phototropism, though the less rapid attainment of the new position in unilaterally illuminated hypocotyls is possibly a reflexion of the reduced rate of growth, and if the inhibition were greater then phototropic curvature might not occur. Even in untreated material phototropic curvature supervenes on geotropic response of hypocotyls suggesting that light is much more potent in establishing unequal distribution of auxin than gravity, and it is possible that (I) is unable to maintain equality of auxin concentration in a unilateral light field.

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- ¹ Jones, R. L., Metcalfe, T. P., and Sexton, W. A., *J. Sci. Food and Agric.*, **5**, 32 (1954).
- ² Audus, L. J., in *Physiology of Plant Growth and Development*, 205 (edit. by Wilkins, M. B., Publisher, 1969).
- ³ Wilkins, M. B., *Arm. Rev. Plant. Physiol.*, **17**, 379 (1966).
- ⁴ Khan, A. A., *Physiol. Plant.*, **20**, 306 (1967).
- ⁵ Mentzer, C., Molho, D., and Pacheco, H., *Bull. Soc. Chim. Biol.*, **32**, 572 (1950).
- ⁶ Vaniček, V., *Ann. Acad. Tchecoasl. Agric.*, **22**, 337 (1950).
- ⁷ Iversen, T.-H., *Physiol. Plant.*, **22**, 1251 (1969).
- ⁸ Cholodny, N., *Ber. Deutsch. Bot. Ges.*, **42**, 356 (1924).
- ⁹ Went, F. W., *Proc. Koninkl. Neo. Akad. Wetenschap. Amsterdam*, **34**, No. 8, 1 (1925).
- ¹⁰ Berrie, A. M. M., Parker, W., Knights, B. A., and Hendrie, M. R., *Phytochemistry*, **7**, 567 (1968).
- ¹¹ Nuttle, G. E., *Plant Physiol.*, **20**, 433 (1945).
- ¹² Mayer, A. M., *Israel. J. Bot.*, **13**, 41 (1964).
- ¹³ Berrie, A. M. M., Hendrie, M. R., Parker, W., and Knights, B. A., *Plant Physiol.*, **42**, 889 (1967).
- ¹⁴ Veldstra, H., and Havinga, E., *Enzymologia*, **11**, 373 (1943/45).

Mitotic Cycles in Dicotyledons and Monocotyledons

THE duration of the mitotic cycle in comparable tissues of different plant species is closely correlated with the amount of nuclear DNA^{1,2}. In a survey of seven species Van't Hof² showed that the average duration of the mitotic cycle in root tip meristems increases at the rate of 0.3 h/pg of DNA. Our own data agree in general with these results, but we have found certain exceptions. For example, where the increase in DNA is brought about by the addition of B chromosomes the duration of mitosis is increased to a disproportionate extent³. We have also found an unexpected difference in the relation between nuclear DNA and the duration of mitosis in monocotyledons as compared with dicotyledons.

Table 1 and Fig. 1 show, as expected, the increasing duration of the mitotic cycles with increasing DNA. They also show that the rate of increase is of the same order in both monocotyledons and dicotyledons. There is in one respect, however, a marked difference between monocotyledons and dicotyledons. For the range of DNA values covered by this survey the complete mitotic cycle is, on average, almost 4 h longer in dicotyledons.

It is pertinent to inquire whether this consistently large difference between the two classes of angiosperms is attributable to any particular phase of mitosis or whether all phases are equally affected. From Table 1 and Fig. 1 it is evident that there is no difference in the duration of the synthesis (S) phase. Neither were there significant differences in the G2 and division phases. From Table 1 it is clear that the difference between monocotyledons and dicotyledons is accounted for largely by the variation in G1, that part of interphase preceding synthesis.

There is evidence that the extension of G1 in dicotyledons may be associated with the degree of chromosome coiling during division. Table 1 shows that the DNA density of chromosomes at metaphase of mitosis in the dicotyledons is almost twice that in monocotyledons, the chromosomes being more highly coiled. It therefore seems reasonable to suggest that in dicotyledons the uncoiling of chromosomes before synthesis would require a longer time and a longer G1 than in monocotyledons. The difference in chromosome organization which distinguishes the two groups may, in turn, stem from one of two genetic causes. The first is a difference in the basic archi-

Table 1 Amount of DNA per Nucleus, Duration of Mitotic Cycles, and G1 and the DNA per Unit Volume of Metaphase Chromosomes in Root Tip Meristems of Diploid Dicotyledons and Monocotyledons

	DNA (2C value) [$\times 10^{-12}$ g]	Mitotic cycle (h)	Synthesis (h)	G1 (h)	DNA per unit volume of chromosomes [pg/ μm^3] at metaphase
Dicotyledons					
<i>Lycopersicum esculentum</i>	3.9	10.6 ²	4.3 ²		
<i>Crepis capillaris</i>	4.2	10.8 ²	3.25 ²		
<i>Linum usitatissimum</i>	1.4	11.2	4.1		
<i>Lathyrus angulatus</i>	8.9	12.25	3.9	4.4	0.400
<i>Lathyrus articulatus</i>	12.4	14.25	4.3	3.9	0.508
<i>Lathyrus tingitanus</i>	18.1	16.75	5.3	6.3	0.574
<i>Lathyrus hirsutus</i>	20.1	18.0	5.0	7.8	0.454
<i>Nigella damascena</i>	21.1	16.5	10.5	1.5	
<i>Vicia faba</i>	23.9	19.3 ⁵	7.3 ⁵	4.9 ⁵	0.430
					Mean 0.473
Monocotyledons					
<i>Lolium perenne</i>	9.9	8.6	4.2	0.5	0.352
<i>Zea mays</i>	11.0	10.5	4.25	0.5	0.364
<i>Secale cereale</i>	18.9	11.7	6.0	1.0	0.178
<i>Allium fistulosum</i>	26.3	15.1	7.1	<0.5	0.194
<i>Allium cepa</i>	33.5	17.8	9.6	<0.5	0.222
<i>A. cepa</i> $\times$ <i>A. fistulosum</i>	29.9	16.6	8.6		
<i>Tradescantia paludosa</i>	38.8	20.0 ⁴	10.8 ⁴		
<i>Hyacinthus orientalis</i>	49.7	24.0	13.6	0.8	0.234
					Mean 0.257

2C DNA values were obtained by Feulgen photometry. Conversion from arbitrary to absolute units was achieved using Van't Hof's 2C value for *Allium cepa*— 33.5×10^{-12} g. The duration of mitotic cycles and of synthesis were estimated by the pulse labelling technique of Quastler and Sherman⁶.

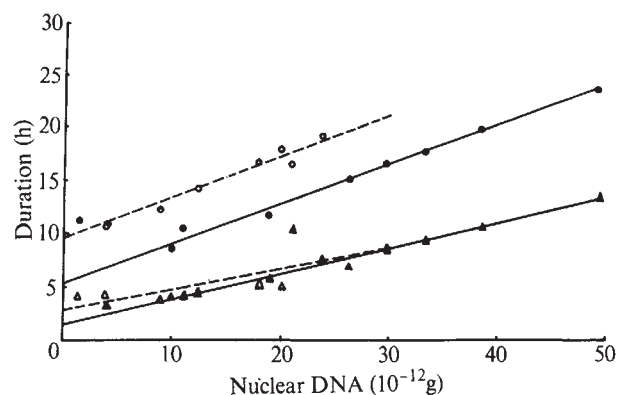


Fig. 1 The duration of the complete mitotic cycle and of the synthesis phase in root tip meristems plotted against the 2C nuclear DNA amount in diploid monocotyledons (—) and dicotyledons (---). ●, ○, Complete cycle; ▲, △, S.

texture of the DNA-protein complex. The second is a genotypic control imposed by the specific activity of one or more genes which regulate the extent of chromosome coiling. There is no means at present of distinguishing between the two possibilities.

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- ¹ Van't Hof, J., and Sparrow, A. H., *Proc. US Nat. Acad. Sci.*, **49**, 897 (1963).
- ² Van't Hof, J., *Exp. Cell. Res.*, **39**, 48 (1965).
- ³ Ayonoadu, U. A. U., and Rees, H., *Exp. Cell. Res.*, **52**, 285 (1968).
- ⁴ Wimber, D. E., *Amer. J. of Bot.*, **47**, 828 (1960).
- ⁵ Evans, H. J., and Scott, D., *Genetics*, **49**, 17 (1964).
- ⁶ Quastler, H., and Sherman, F. G., *Exp. Cell. Res.*, **17**, 420 (1959).

Importance of Synchrony of Ear Emergence in Plant Breeding Programmes

IN plant breeding, there is a general trend to apply the term "synchrony" to intra-plant variance or inter-tiller variance of a character like ear emergence. Thus, plants with the least intra-plant variance are considered more synchronous. The importance of synchrony of tillering in breeding programmes and the procedure for studying it is not yet well defined. To

Table 1 Correlation Coefficients between Grain Yield per Plant and the Intra-plant Variance (Synchrony) of the Four Component Characters Studied

Variety	Correlation coefficients between grain yield per plant and			
	Synchrony of ear emergence	Synchrony of height	Synchrony of the number of grains per ear	Synchrony of weight of grains per ear
'Archer'	-0.14	-0.39	-0.59	-0.71 *
'Impala'	0.79 †	0.17	0.51	0.60
'Proctor'	0.21	-0.07	-0.46	0.43
'Rika'	-0.49	-0.52	-0.69 *	-0.21
'Spratt'	0.46	-0.24	-0.20	0.21
'Sultan'	-0.18	-0.17	-0.22	-0.10

* Significant at the 5% level.

† Significant at the 1% level.

investigate this topic further I have studied six genetically distinct varieties of spring barley which have been bred in the past with little or no evidence of conscious selection for synchrony.

A randomized block design comprising five replications was used for laying out the field experiment in 1969. Observations on grain yield per plant, days to ear emergence, plant height, the number of grains per ear and weight of grains per ear were recorded for each individual tiller of a plant. Individual plants were spaced 5 cm apart in normal field conditions. Two approaches were used to study synchrony, one using intra-plant variance and the other using regression coefficients of the mean value of a particular tiller on its own tiller number. The tiller numbers were given in the order of their ear emergence. In the absence of a more appropriate term, synchrony has been used to describe characters other than ear emergence to reflect intra-plant variance, the higher synchrony denoting least intra-plant variance.

The correlation coefficients between grain yield per plant and the intra-plant variance used as synchrony of the four characters studied are given in Table 1 for each variety. If the synchrony of ear emergence was negatively correlated with grain yield (as would be desirable if the genotypes were higher yielding and had least intra-plant variance for ear emergence), then the association was also negative between the synchrony of other characters and grain yield per plant. Of the six varieties studied only 'Rika', 'Sultan' and 'Archer' have shown this trend, and the intra-plant variance for all the characters is also low. Moreover, the synchrony of all the characters in three of the six varieties seems to be positively associated with the synchrony of ear emergence.

The regression slopes obtained by regressing the mean value of a particular tiller on its own tiller number can be used to judge the synchrony of a variety for the character in question. The idea is that the subsequent tillers of a synchronous variety should show phenotypic expression of a particular character which is similar, if not identical, to that of the first few tillers. Thus the genotype showing the least or no regression slope is

Table 2 Regression of the Mean Value of each Individual Tiller on its Number for Different Characters estimated for Six Varieties of Spring Barley

Variety	Ear emergence (days) $b \pm S_b$	Height (cm) $b \pm S_b$	No. of grains/ear $b \pm S_b$	Weight of grains/ear $b \pm S_b$
'Archer'	1.301 ± 0.111	-1.769 ± 0.345	-0.654 ± 0.144	-0.068 ± 0.012
'Impala'	1.461 ± 0.025	-2.190 ± 0.128	-1.100 ± 0.045	-0.069 ± 0.004
'Proctor'	1.510 ± 0.043	-1.883 ± 0.209	-0.912 ± 0.097	-0.066 ± 0.005
'Rika'	0.698 ± 0.061	-0.717 ± 0.144	-0.388 ± 0.065	-0.043 ± 0.004
'Spratt'	1.429 ± 0.057	-3.425 ± 0.318	-1.106 ± 0.145	-0.099 ± 0.011
'Sultan'	1.095 ± 0.085	-1.752 ± 0.120	-0.795 ± 0.076	-0.061 ± 0.004

b, Regression coefficient; S_b , standard error of b.

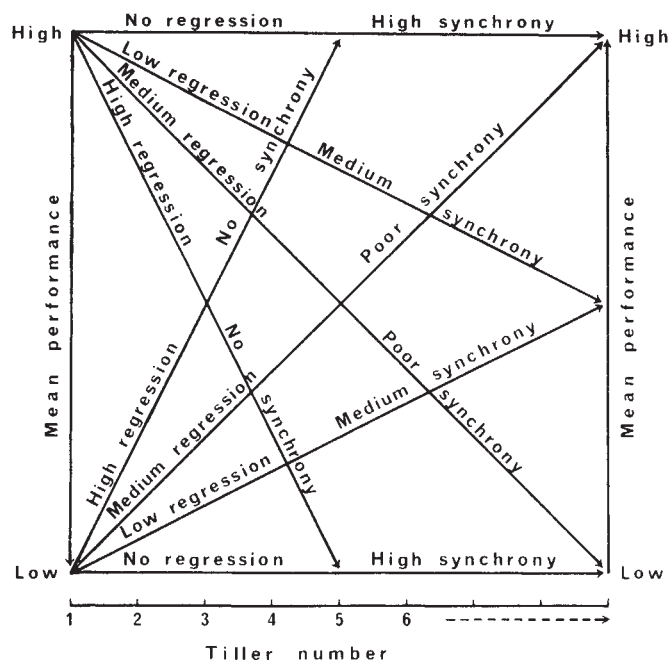


Fig. 1 A generalized scheme for studying synchrony.

the most synchronous. Accordingly, a generalized scheme for studying synchrony is presented in Fig. 1. A two-way approach is presented, where characters like ear emergence and height are recorded in ascending and descending order, respectively. Thus, the sign of the regression slope is not relevant, and it is the magnitude of the slope which determines synchrony. As Table 2 shows, 'Rika', 'Sultan' and 'Archer' expressed least regression slopes for the four characters studied. 'Rika' had the lowest regression slopes and was the most synchronous, whereas 'Sultan' and 'Archer' expressed medium synchrony. 'Spratt' was the most non-synchronous variety.

'Rika', 'Sultan' and 'Archer', which showed synchrony of ear emergence and other characters, might well be exploited. It would be desirable, however, to confirm the trend in a wide range of environments and the genetic basis of synchrony needs careful investigation. Of the two approaches used for studying synchrony, that involving regression coefficients is simpler and more informative and could be used in the future.

Reports of the effectiveness of the component approach to selective breeding are becoming available for cereal crops such as wheat¹⁻⁴ and barley^{5,6}. These studies have provided considerable evidence in favour of component breeding and it has been established that selection on the basis of grain weight without ignoring grain yield would lead to improvements. I have now found that synchrony of tillering, which can be judged at the stage of ear emergence or even earlier in plant development, could also be used as a selection criterion for improving yield in plant breeding programmes.

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¹ Borojevic, S., and Cupina, T., *Proc. Third Intern. Wheat Genet. Symp.*, Canberra, 388 (1968).

² Borojevic, S., and Cupina, T., *Contemporary Agriculture-Novi Sad*, 17, 5 (1969).

³ Paroda, R. S., and Joshi, A. B., *Heredity*, 25, 383 (1970).

⁴ Paroda, R. S., and Joshi, A. B., *Indian J. Genet.*, 30, 298 (1970).

⁵ Rasmusson, D. C., and Cannell, R. Q., *Crop Sci.*, 10, 51 (1970).

⁶ Borthakur, D. N., and Poehlman, J. M., *Crop Sci.*, 10, 452 (1970).

Evaluation of Bicuculline as a GABA Antagonist

THERE has been controversy recently about the reliability of bicuculline as an antagonist of the putative inhibitory transmitter γ -aminobutyric acid (GABA) in the brain¹⁻³. Although the effects of bicuculline have so far been explained in terms of its combination with the GABA receptor, bicuculline might also affect the removal processes for GABA and the synthesis and release of endogenous GABA. We have therefore made quantitative analyses of the effects of iontophoretically applied bicuculline on the responses of neurones to GABA and have also estimated the effects of bicuculline on the concentration and turnover of GABA in the brain, GABA uptake and the synthetic and degradative enzymes glutamate decarboxylase (GAD) and 4-aminobutyrate : 2-oxoglutarate aminotransferase (GABA-T).

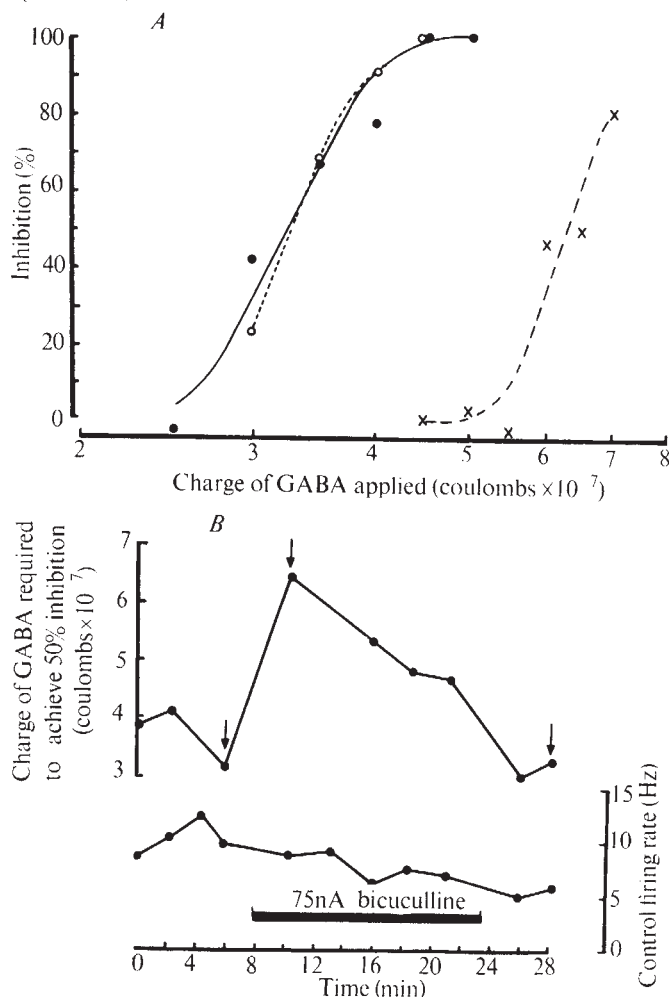


Fig. 1 A, Dose-response curves of percentage inhibition of cell firing against coulombs of GABA applied at a constant current of 10 nA. The cell was 1.33 mm deep in the suprasylvian cortex of a cat and was driven by continuous application of 75 nA glutamate. ●, Control response; x, response during continuous application of 75 nA bicuculline; ○, recovery response. B, Plot of coulombs of GABA required to achieve 50% inhibition of the firing rate of the same cell as above during repeated 10 nA applications of GABA before, during and after a 75 nA application of bicuculline. The arrows indicate the values taken from the curves in A. Bicuculline clearly antagonized the action of GABA but had no effect on the control firing rate of the cell.

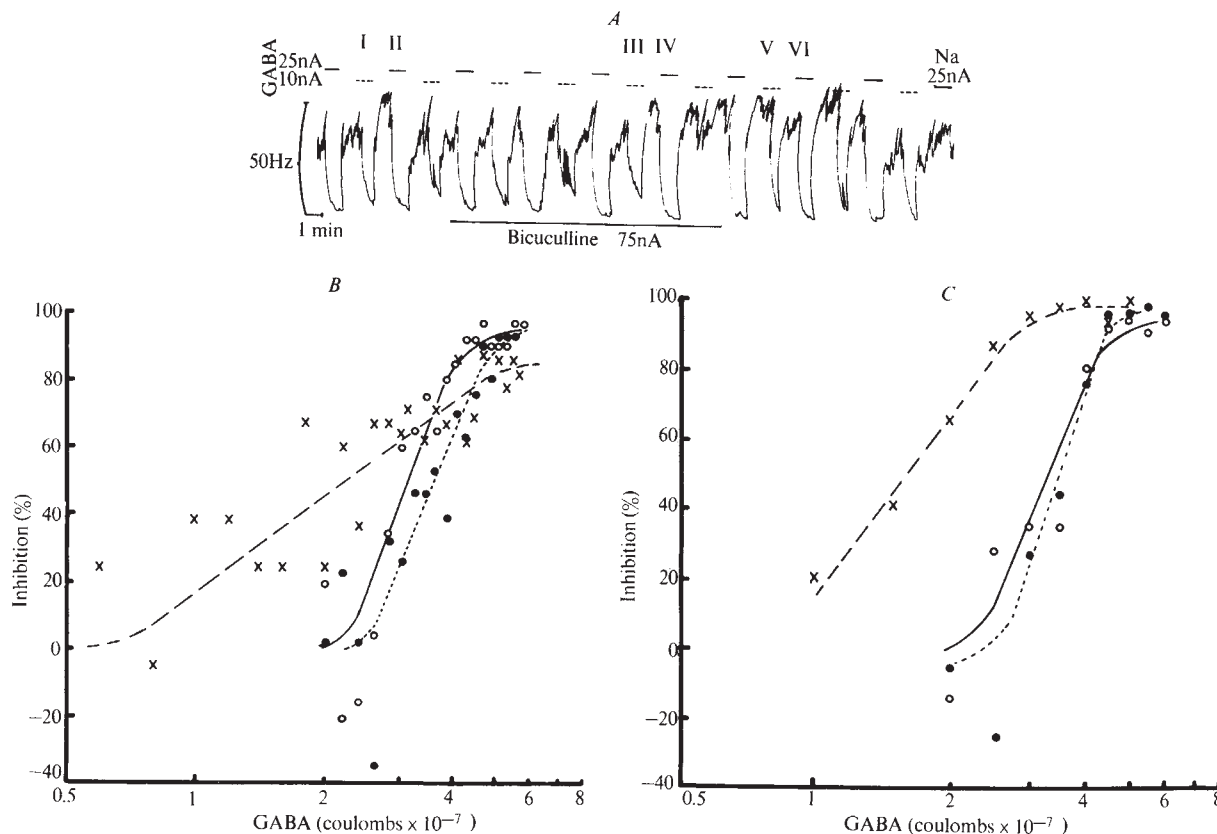


Fig. 2 *A*, Analogue rate-meter record of the firing of a cell driven by continuous application of 25 nA glutamate at a depth of 1.82 mm in the suprasylvian cortex of the cat. GABA 10 nA and 25 nA was applied as indicated by the bars above the trace. Bicuculline 75 nA was applied continuously during the period indicated by the bar below the trace. Responses labelled I–VI are plotted in the form of dose-response curves in *B* and *C*. *B*, Responses I (—○—), III (—×—) and V (—●—) plotted as percentage inhibition against coulombs of GABA applied at a constant current of 10 nA. Responses were calculated from the interval-time spike counter record (not shown) which was obtained simultaneously with the rate-meter trace shown above. *C*, Responses II (—○—), IV (—×—) and VI (—●—) to a constant current application of 25 nA GABA plotted as in *B* above.

In iontophoretic experiments, sixty cortical cells have been studied in twelve cats anaesthetized with 50% nitrous oxide in oxygen and 1% halothane. Drugs were applied and extracellular spike potentials recorded through standard five and seven-barrelled glass micropipettes with tip diameters 5–7 μ m as in previous studies⁴. The extracellular spike potentials were counted with an interval-time spike counter and also displayed on an analogue rate meter. Cells which did not fire spontaneously were driven by continuous application of glutamate or DL-homocysteate. The inhibitory effects of GABA were analysed by constructing dose-response curves of percentage inhibition of cell firing against the coulombs of GABA passed.

We have confirmed the experience of Curtis *et al.*¹ that bicuculline can reversibly antagonize the depressant effect of GABA on cortical neurones (for example, Fig. 1). This was not a consistent finding, however, for, in common with

Godfraind *et al.*² we sometimes found that the response to GABA was potentiated in the presence of bicuculline. For example, in the experiment shown in Fig. 2, we applied two currents of GABA alternately. Inspection of the analogue rate-meter trace (Fig. 2*a*) shows that the response to 10 nA GABA was eventually blocked after about 15 min continuous application of bicuculline, whereas the response to 25 nA GABA was not blocked. Closer inspection of the trace, however, reveals that the onset of the response to both currents of GABA was accelerated throughout the application of bicuculline. This potentiation is more clearly shown in the dose-response curves plotted for certain of the GABA applications in Figs. 2*b* and *c*. The effects of bicuculline were completely reversible and indicate that it can both potentiate and antagonize GABA on the same cell, depending on the relative concentrations of the two substances.

In ten cells (five spontaneous, five driven), the responses to

Table 1 Effect of Bicuculline on the Rate of Disappearance of ³H-GABA from the Cerebellum, Cerebral Cortex and Whole Brain of the Rat

Area	Route	Dose	<i>t</i> _{1/2} h between 2–8 h (95% confidence limits)		P value
			Control	Bicuculline	
Cerebellum	i.c.	12.5 μ g	1.25 (1.00–1.50)	2.95 (2.49–3.41)	<0.05
Cerebral cortex	i.c.	12.5 μ g	3.21 (2.75–3.71)	8.40 (7.62–8.98)	<0.02
Whole brain	i.p.	2.5 mg/kg	2.88 (2.57–3.19)	4.30 (4.09–4.59)	<0.05
	i.c.	12.5 μ g		3.68 (3.36–4.00)	<0.02

³H-GABA (10 μ Ci) was injected intracisternally (i.c.) immediately after the injection of bicuculline. The animals were killed at times between 2 and 8 h after injection and the ³H-GABA remaining in the brain was measured. From the regression of log. ³H-GABA concentration on time, *t*_{1/2} was calculated by the formula: *t*_{1/2} = (log 2)/slope. In whole brain of rats injected with bicuculline 2.5 mg/kg intraperitoneally (i.p.) 2 h after injection, the total ³H-GABA content was 78,260 $\pm$ 6,382 d.p.m. (mean $\pm$ s.e.) which was not significantly different from control rats at 2 h, 62,320 $\pm$ 5,820 d.p.m. Controls were injected with an appropriate volume of solvent.

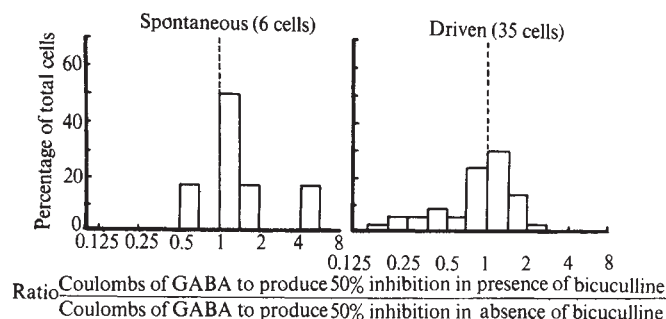


Fig. 3 Frequency distribution of the effect of bicuculline on the responses of cat cerebral cortical cells to GABA. A quantitative estimate of the effect of bicuculline was obtained from the ratio of the coulombs of GABA required to give 50% inhibition of the firing rate of a cell in the presence and absence of bicuculline. A ratio of more than 1 indicates antagonism of GABA and a ratio of less than 1 indicates potentiation.

GABA were sufficiently antagonized by bicuculline such that the inhibition due to the selected current of GABA failed to reach more than 80% in the presence of bicuculline. In a further forty-one cells, more quantitative estimates of the effects of bicuculline were made. In these cells, lateral shifts in the dose-response curves could be measured since the applied current of GABA was sufficient eventually to cause complete inhibition of firing in the presence of bicuculline. An analysis of the shifts in the dose-response curves (Fig. 3) reveals that bicuculline had a greater tendency to antagonize the effects of GABA than to potentiate them in cells which were spontaneously active. In cells driven by glutamate or DL-homocysteate, there seemed to be an approximately equal occurrence of potentiation and antagonism of the action of GABA in the presence of bicuculline.

The effects of bicuculline on the enzymes responsible for the synthesis (GAD) and degradation (GABA-T) of GABA were studied in rats, using the assay techniques of Roberts and Simonsen⁵ and Hall and Kravitz⁶, respectively. There was no significant effect of bicuculline on the activity of glutamic acid decarboxylase (GAD) in brain homogenates exposed to 1 mM bicuculline *in vitro* and no effect was seen in brain homogenates prepared from rats which had been previously injected with bicuculline, 2.5 mg/kg intraperitoneally or 12.5 µg intracisternally. There was a small inhibitory effect of bicuculline on the activity of GABA-T in brain homogenates exposed to bicuculline (1 mM) *in vitro*, the activity of the enzyme being 82% ($P < 0.02$) of the control value. It was not possible, however, to show any inhibition of GABA-T in brain homogenates prepared from rats which had been previously injected with subconvulsive doses of bicuculline (2.5 mg/kg intraperitoneally or 12.5 µg intracisternally) nor did these doses of bicuculline have any significant effect on the endogenous GABA concentration in the brain, as measured by the method of Jakoby and Scott⁷.

Since brain tissue has a powerful uptake mechanism for GABA⁸, the action of GABA released from nerve terminals or applied iontophoretically onto neurones may be terminated by re-uptake into nervous tissue. Bicuculline appears to have no effect on this mechanism since the uptake of ³H-GABA by cortical slices from rat brain exposed to bicuculline (0.14 mM) was not significantly different from that in controls.

To obtain some idea of the effect of bicuculline on GABA turnover, a tracer dose of ³H-GABA was injected intracisternally into the rat and the rate of disappearance of ³H-GABA from the brain was followed⁹. Subconvulsant doses of bicuculline significantly increased the half-life of ³H-GABA in the brain between 2 and 8 h after injection (Table 1). The total amount of ³H-GABA in the brains of the bicuculline treated animals 2 h after injection was not significantly different from controls, suggesting that the half-life of ³H-GABA during the first 2 h was not altered. The interpretation of these results is difficult at present, as the

functional significance of the two phases of ³H-GABA disappearance from the brain is not yet understood. The increased half-life of ³H-GABA between 2 and 8 h is unlikely to be due to inhibition of GABA-T since this effect was not seen in animals treated with AOAA (Collins and Neal, unpublished results). It is possible that bicuculline affects the neurally evoked release of GABA from nervous tissue *in vivo*, although the spontaneous efflux of ³H-GABA from slices of rat cortex is unaffected by incubation with bicuculline (1 mM) for periods up to 1 h.

We have shown that iontophoretically applied bicuculline has more than one effect on the responses of cortical neurones to GABA. The potentiating effect of bicuculline is unlikely to be due to its weak inhibitory action on GABA-T or to inhibition of uptake. Other workers¹⁰, however, have found that bicuculline can potentiate the effect of stimulating inhibitory pathways and have explained this as antagonism of an inhibitory influence on the inhibitory pathway being stimulated. A similar explanation for the potentiation of iontophoretically applied GABA does not seem to be feasible and the possibility remains therefore that this could be due to a direct non-specific effect of bicuculline on the post-synaptic membrane.

The antagonistic effects of bicuculline on the responses to GABA are probably due to a post-synaptic action, but a pre-synaptic component cannot yet be completely excluded in view of our results on GABA turnover. The potency of bicuculline as an antagonist is low, less than a doubling of the amount of GABA applied being required to overcome the antagonism. This should be compared with a ratio of 3.4 in the case of glycine and strychnine¹¹ and a very much larger ratio still in the case of acetylcholine and atropine (Clarke, Davies and Straughan, unpublished results). Thus, it appears that locally applied bicuculline is not a potent or reliable enough antagonist of GABA in the cerebral cortex to be used for confident identification of GABA-operated synapses or evaluation of the physiological role of GABA in that part of the brain.

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1. Curtis, D. R., Duggan, A. W., Felix, D., and Johnston, G. A. R., *Nature*, **226**, 1222 (1970).
2. Godfraind, J. M., Krnjević, K., and Pumain, R., *Nature*, **228**, 675 (1970).
3. Curtis, D. R., Duggan, A. W., Felix, D., and Johnston, G. A. R., *Nature*, **228**, 675 (1970).
4. Johnson, E. S., Roberts, M. H. T., and Straughan, D. W., *J. Physiol.*, **203**, 261 (1969).
5. Roberts, E., and Simonsen, D. G., *Biochem. Pharmacol.*, **12**, 113 (1963).
6. Hall, Z. W., and Kravitz, E. A., *J. Neurochem.*, **14**, 45 (1967).
7. Jakoby, W. B., and Scott, E. M., *J. Biol. Chem.*, **234**, 937 (1959).
8. Iversen, L. L., and Neal, M. J., *J. Neurochem.*, **15**, 1141 (1968).
9. Collins, G. G. S., and Neal, M. J., *Brit. J. Pharmacol.*, **41**, 396 (1971).
10. Duggan, A. W., and McLennan, H., *Brain Res.*, **25**, 188 (1971); Felix, D., and McLennan, H., *Brain Res.*, **25**, 661 (1971).
11. Johnson, E. S., Roberts, M. H. T., and Straughan, D. W., *Brit. J. Pharmacol.*, **38**, 659 (1970).

BOOK REVIEWS

Berzelius: His Life

Jacc Berzelius: His Life and Work. By Erik J. Jorpes. Translated from the Swedish by Barbara Steele. Pp. 156. (University of California: Berkeley and Los Angeles, 1970.) n.p.

THIS is an unimaginative, but useful, book which will be of value principally as a summary of what is already familiar to students of the chemistry of the first half of the nineteenth century, but is not readily available in a single small volume. The author describes Berzelius's life as a succession of exercises, with little attempt to put any particular piece of work into a historical context, so that one is left with a feeling of: "So that is how it looked to Berzelius day by day, but what was his life really all about?"

The story of any man's life is always a story about something else as well, and Berzelius's life was really all about a great revolutionary movement in human thought (roughly contemporary with the French Revolution and its aftermath, but more far reaching in its effects on mankind as a whole). This was the great advance in human thought which brought quantitative considerations into man's knowledge and control of the material substances of his environment. But Dr Jorpes is no philosopher of chemistry. (Indeed, do we have one today? How useful for us all if there were.) He prefers to present lists of Berzelius's work in each of his several practical fields. His theoretical work and the important controversies it aroused get short treatment and even that without much comment, so we get very little justification for Berzelius's reputation in the eyes either of his contemporaries or of his modern critics.

Perhaps Dr Jorpes, being a countryman of Berzelius, sees him close to, as a fellow medical man, as a fellow member of Swedish institutions, and so finds no need to draw a heroic portrait. Here we see the main value of this book. Most studies of Berzelius in languages other than Swedish have necessarily concentrated on his great influence, and, seeing this to be very great, have given us small flat portraits of a genius. Dr Jorpes gives a larger, rounder picture of a plain, likable, modest, determined, sensible man, sometimes witty, sometimes preoccupied, sometimes sick and sad, magnanimous in his private life even if he could be, on uncharacteristic

occasions, grudging and resentful in his public scientific life. In his middle age he was man enough to attract as his wife a much younger woman above his station, and weak enough to be swayed by her to dismiss the phenomenal Anna, cook, housekeeper and laboratory assistant.

Dr Jorpes hints here and there at aspects of Berzelius's life which would be worth extended study. He was very conscious of the problems of communication. He knew the linguistic obstacles for even a widely travelled Swede, yet he was aware that this was far less of an obstacle to scientific development than the insularity of attitude of which he accused English chemists, not excluding Davy.

In Berzelius's works we can see the dual role of mineralogy both pulling chemistry along the road of quantitative systematics, and yet hampering its progress towards a grasp of the importance of internal structure. Someone might investigate this. And we have not yet discerned all the differences between what electricity meant to chemists and to physicists in Berzelius's time.

To some extent he was the man of a book, spending a great deal of time that might have been spent in the laboratory in writing and revising a compendious textbook. Although much translated and widely used, it seems to have had far less influence than his novel year-books, which set the pattern for those progress reports which kept scientific controversy active and wide ranging when communication was slow.

His epitaph could be written in a few technical terms like isomerism and catalysis, but any chemical formula would serve just as well, for it was he who devised the literal notation which gave chemistry H_2O and $CaCO_3$, and all the intelligibility such formulae imply.

As a supplement there is reprinted (in German) the summary of Berzelius's analytical work during 1808–21, which H. G. Söderbaum originally gave in his Kahlbaum Monograph, *Jac. Berzelius' Werden und Wachsen* (Leipzig, 1899). This, more than any mere description, shows Berzelius's enormous productivity in his best years.

The book is aptly illustrated and well indexed. The translator seems to be as competent in the subject matter as she is in the language of the original.

F. GREENAWAY

Chemical Tribute

Chemical Dynamics: Papers in Honor of Henry Eyring. Edited by Joseph O. Hirschfelder and Douglas Henderson. (Advances in Chemical Physics, Vol. 21.) Pp. xxxiii+816. (Wiley-Interscience: New York and London, April 1971.) £10.75.

THE reviewer of this volume is in something of a quandary. Should he review Henry Eyring the man, Henry Eyring the scientist, the contributions of Henry Eyring or the contributions to the volume under review? Convention would dictate the last of these four possibilities. But the reviewer craves the indulgence of the readers while he says a word about the other three. In an age when society, and in particular the young, have shown a degree of revulsion from science, the question might well be asked whether or not this is due to the manner in which it is often presented—cut and dried, pre-packaged and apparently untouched by human hands. One speculates whether science should not be presented as a living thing, made by human beings subject to all the frailties of the species. Rarely is a paper presented to show the often haphazard lines along which the work developed. Only the crystal clear logic of the final analysis is presented, cold and remorseless. This is not science as most practitioners know it. A book like the present volume goes a long way to restore humanity to science, in the many glimpses we are given by the various contributions of Henry Eyring the man, the teacher and the scientist. And the picture that emerges is not only that of a great scientist, but of a great human being.

It would be wrong to suggest that all the papers will be of interest to everyone. Indeed, the wide spectrum covered is yet another tribute to the catholicity of interest of Henry Eyring. The papers are divided into six sections: molecular quantum mechanics (edited by A. A. Frost); theory of reaction rates (edited by K. J. Laidler); properties of molecules (edited by W. Kauzmann); theory of liquids (edited by D. Henderson); biological applications (edited by F. H. Johnson), and engineering applications (edited by C. J. Christensen). As might be imagined, the largest section is that dealing with the theory of reaction rates, which consists of fourteen varied contributions spanning a wide

area from the mechanism of electron energy transfer from excited mercury atoms to paraffins, to the theory of non-Newtonian flow. The second largest is the theory of liquids, which ranges from molecular beam studies (as a means of evaluating intermolecular potentials) to the theory of plastic crystals. The sections on molecular quantum mechanics and properties of molecules overlap somewhat, as might be expected. Biological applications also cover a wide area, from protein conformations to the dynamics of life. No less varied are the engineering applications, which include the synthesis of diamond and the theory of detonation.

As will have been observed from what I have said, the volume accurately mirrors the scientist in honour of whose seventieth birthday it was produced. The man-made terms, mathematician, physicist, chemist, biologist, are too narrow to describe Henry Eyring. In browsing through this volume, the reader is brought face to face with a scientist, a man dedicated to enlarging the understanding of the working of nature, using techniques drawn from many disciplines. And this may well prove an inspiration to the young worker who feels that in the past he has been confined within a rigid but arbitrary discipline. Few scientists, whatever their interest, could fail to benefit from recourse to this volume.

ALLAN MACCOLL

Science and Indian Development

Science in India: Institution-Building and the Organizational Systems for Research and Development. By Ward Morehouse. Pp. xvi+144. (Popular Prakashan: Bombay, 1971.) Rs 25.

Physics in India: Challenges and Opportunities. (Proceedings of the Conference on Physics, Education and Research, June 1970.) Pp. iv+323. (National Council for Science Education: New Delhi, 1971.) Rs 10; \$4; £1.25.

THE recent interest in the social implications (both good and bad) of science and technology has reached its culmination in what may well be the most significant social problem of all—overshadowing such currently fashionable fields as environmental pollution, the arms race and technological unemployment—that is, the possible use of Western techniques in helping to resolve the problem of chronic poverty endemic in well over two-thirds of the world's population. The argument runs as follows: since one of the principal factors influencing the very rapid rise in the

standard of living of the Western "developed" nations has been the effective application of techniques derived from advances in the natural sciences to the productive process, it is necessary for the underdeveloped world to make every effort to foster science so as to achieve the same ends. Couched in such simplistic terms the process seems easy, but in practice the transplantation of what is to a large extent an alien social process, in spite of colonialism, has proved fraught with difficulties and has in some cases helped to exacerbate those symptoms commonly associated with underdevelopment, for example open and disguised unemployment, urban squalor and neglect of the land. Clearly the field is ripe for research.

Ward Morehouse's *Science in India* is a preliminary account arising out of a research programme currently being carried out at the Administrative Staff College, Hyderabad, on the organization of science and technology in India. The book has two principal aims. First, to provide the beginnings of a theoretical framework which will act as a basis for assessing the impact of scientific organizations on Indian development. Second, to provide an empirical account of the organizational structure of Indian science.

Taking the second aim first, Dr Morehouse has given us a valuable summary of the various bodies connected with Indian science, their respective functions, history, modes of finance, links with the socio-economic environment and relationships with the political power structure. For anyone intending to work in this field, his account must be necessary reading, drawing together, as it does, a wide variety of material from disparate sources. The author's attempts to provide a theoretical framework, however, are rather weaker not so much for what is said but for what is omitted. According to Dr Morehouse a scientific "organization" will only be effective in contributing to development once it has become an "institution". How shall we know when it becomes an institution? Well, when it begins to interact meaningfully with the socio-economic environment and evolves a cohesive dynamic of its own. This type of functionalism, besides evincing partial circularity of reasoning, does not seem to hold much promise for the resolution of the many problems which characterize Indian science today. In particular it does not give us criteria for deciding when a scientific "institution" is not helping development. Nor is there more than a cursory attempt made to define what is meant by "development".

These criticisms do not attach so much to the proceedings of a recent conference on physics education in India (*Physics in India: Challenges and*

Opportunities). This conference, sponsored by Indian and American governmental and educational institutions at Srinagar, set out to discuss the principal problems connected with physics teaching and research in India and how these might be resolved. The report consists of a series of papers which stress the need for curricular reform in schools and universities, improved laboratory and library facilities, and a more rational use of teaching staff. In addition the need for education to be more broadly based and more intimately related to developmental objectives is emphasized and there are a number of interesting papers on the brain drain and unemployment of graduates, especially that by Parthasarathi. Again this volume is probably of chief interest to the specialist but could usefully be consulted by those concerned with wider aspects of social and economic development.

NORMAN CLARK

Swansea Survey

Swansea and Its Region. Edited by W. G. V. Balchin. Pp. xx+391+58 plates. (Swansea University: Swansea, 1971.) £2.50.

THIS is the traditional volume prepared on the occasion of the annual meeting of the British Association for the Advancement of Science, in this case at Swansea. A similar volume was prepared for the meeting at Cardiff in 1960 and the two together, although a decade apart in time, provide a South Wales survey.

During the past decade the BA handbooks have grown and the present volume is about twice the size of that prepared for the Cardiff meeting. It also has had the advantage of a geographer editor who is experienced in thinking in regional terms. In a review of this length it is impossible to give attention to each of the twenty-five chapters, even if any one person could be competent to assess them individually. It is to the general structure of the volume, therefore, that these comments must be directed although this means that many excellent, individual contributions may appear to be ignored.

In its general make-up the volume suffers from the nature of its origins. Presumably the earliest productions were meant to be simple guide books to the host cities and their environs, but they have evolved into full scale regional surveys. Unfortunately the handbooks fail to throw off the old role as guides and also a commitment to represent most of the sections of the association. The result is that the volume is not a true regional survey but a hybrid product. The contributions to

this volume fall into three categories. The first reflects the old guide book character where local institutions are enumerated and described. Perhaps the chapter on the education service is the best example, for it is a straightforward account of facilities, but with no mention of problems. Have the new comprehensives avoided becoming one-class neighbourhood schools? What are the problems posed by the Welsh language? Indeed, is it taught at all, for it is not mentioned? Is it compulsory and to what level? Also in this same category is the account of the University College, and that of the City of Swansea, which is very far removed from modern urban studies. The second category is the "review of research" approach which devotes its space not to the region *per se* but to the research carried on in the locality. No chapter falls completely into this category, although that on zoology comes nearest to it. One could also place within it the very oddly located part five of the book, which deals with the Lower Swansea Valley Project. This important piece of work certainly merits discussion but it appears as an apparent climax at the end of the volume whereas it might have been more appropriately linked with "conservation". The third type of chapter is that of regional description and interpretation, directed to the local area itself. This quite properly forms the bulk of the book and is admirably done. Even so there is a lack of balance in that social structures are dealt with most briefly in some twelve pages as against some one hundred and twenty on the natural environment.

It is in this general area that I would make my basic criticism. The anthracite area of the coalfield, although not the whole of the Swansea region, has a very distinctive "character". Perhaps the basic feature is that it is the only part of Wales which is both industrialized and substantially Welsh speaking, a point which is never clearly made. It is all the greater pity that no attempt was made to demonstrate these characteristics when the person who could do it best, Professor T. J. Morgan, contributes but a brief chapter on place names, although according to the programme he presented a paper to section H of the association on the peasant culture of the Swansea Valley. This is, after all, to Welshman the land of Gwenallt, but none of that seeps into the book.

There are two further points which are disconcerting. The first is that no attempt is made to define the Swansea region, particularly in terms of its metropolitan structure. It is understandable that, for example, geologist and economic geographer might not be content with the same bounds, but it is

a little odd to find the chapter on climate, exclusively concerned with Swansea itself, while that on botany includes the whole of Carmarthenshire and south-west Breconshire. This at a point where interrelations might be expected. The other disconcerting feature is that there is no direct urban survey of Swansea itself, no growth plan, no land use map, no study of "social areas". This survey of the city has been a conventional feature of nearly all the handbooks and it is certainly not lack of ready material which is the reason.

But perhaps my strictures are unfair. Critics too frequently demand a volume different from the one before them or ask for extensive and, for practical reasons of cost and bulk, impossible additions to the existing volume. If this were a coherent regional survey there is much that one could cut out from this volume and much that would need to be added: but of course it is not, it is a British Association handbook and is thereby committed. Perhaps a conclusion is that the purpose of the BA handbook be reviewed. I conclude, therefore, by reiterating my comments on the high quality of the work that has gone into this book. The editor writes in his introduction that no recent survey of the Swansea area exists and I am sure that many people, for many different purposes, will have recourse to the material contained in *Swansea and Its Region*.

H. CARTER

Caloric Theory

The Caloric Theory of Gases from Lavoisier to Regnault. By Robert Fox. Pp. xiv+378. (Clarendon: Oxford; Oxford University: London, May 1971.) £5.

EDWARD FRANKLAND's comment that "it is by no means necessary that a theory should be absolutely true in order to be a great help to the progress of science" is now a commonplace among historians of science. It is one of the chief purposes of this detailed and technical study to demonstrate the sophistication and complexity of the caloric theory, at least as far as the gaseous state was concerned, and to reveal how successful its conceptual framework proved. The notion that caloricists were wrong headed and obscurantist was a late-Victorian view developed, understandably enough, in the wake of thermodynamic triumphs. In part, it was a Royal Institution saga devised by John Tyndall in praise of its founder Count Rumford. But, as Dr Fox cogently argues, Rumford appears in the history of caloric theory only by default since his experiments were unoriginal and "off the point", for they

were directed only against the weaker Irvinist model of heat rather than against the stronger continental theory of Lavoisier and Laplace. Indeed, the latter's correction of Newton, and absorption of adiabatic phenomena in the derivation of the velocity of sound in 1816, two years after Rumford's death, was a major triumph for the caloric theory of gases.

The basic assumption of the caloricists from the 1770s onwards was that gas particles were held in stationary (not vibratory) positions by repulsive forces between them which were produced by the presence of an imponderable fluid of heat which either surrounded, or fitted between, the gas particles. Heat itself, like ponderable matter, was conserved. This model was basically Newtonian, modified by the discoveries of pneumatic chemists, and fused with eighteenth century views on fire. However, Dr Fox's book is more than an internalist study of an outmoded theory and its experimental justifications. His own interest is obviously in the character of nineteenth century French science, particularly in the general scheme of scientific investigation he calls "Laplacian", and whose institutional basis at Arcueil was recently explored by Dr Crosland. According to this research programme, all physical phenomena were to be explained in terms of short range attractive and repulsive forces. This general interpretation enables the author to highlight and contrast the activities of the mathematical Laplace, the engineering-orientated Carnot, and the arch-caloricist Avogadro who, in Italian isolation, remained tragically unaware of his work's discredibility to both physicist and chemist.

The author attributes the downfall of the caloric theory to a complex of causes, including the rise of Berzelian electrochemistry and the Fresnelian wave theory of light. Readers will find the pertinence of these new theories are too briefly analysed in contrast to the main contention that the most significant cause was philosophical and institutional: "an overall change of the style of French science between 1815 and 1830" which was anti-Laplacian. Comte's positivism reflected this change of mood, while the failure of Dulong and others to find viable alternative theories in the 1820s and 1830s led to widespread agnosticism towards theories of heat and atomism except as pedagogic aids. Regnault's brilliant, but tedious, experimental work is seen as a symbol of the decline of French theoretical physics. It follows, as Dr Fox plausibly argues, that the rise of the kinetic theory of gases in Britain and Germany during the 1850s was almost completely unrelated to the downfall of caloric theory which had effec-

tively occurred two decades before.

This is an important monograph and deserves to be read for the historical questions it raises about nineteenth century science as much as for its definitive account of a forgotten physical theory.

W. H. BROCK

Analytical Geochemistry

Analytical Geochemistry. By Lord Energlyn and L. Brealey. (Methods in Geochemistry and Geophysics, Vol. 5.) Pp. xv+426. (Elsevier: Amsterdam, London and New York, 1971.) £9.25.

THIS book attempts to describe a wide variety of chemical and physical analytical procedures of use to geochemists. While chemical methods are described in some detail the treatment of physical methods is disappointing, and out of date in many instances.

The introduction contains a curious selection of geochemical topics. Igneous and metamorphic geochemistry are discussed only in terms of classification, with details of the well established "CIPW norm" and "Niggli equivalent norm". Although hydrothermal processes are briefly mentioned there is virtually no reference to modern work, even to such excellent texts as *Geochemistry of Hydrothermal Ore Deposits* edited by H. L. Barnes. A very brief, dated section on chemical solubility products and an introduction to fluidization phenomena are followed by interesting observations on the role of organic solvents in sedimentary geochemistry. The latter topic reflects the senior author's interests, and includes considerable reference to his own work.

The second chapter outlines the principles of qualitative analysis of rocks and minerals and is particularly noteworthy for the detailed description of the little known technique of membrane colorimetry. This technique incorporates a variety of methods for the transfer of chemical images from flat surfaces of metal, rocks and plants.

Quantitative chemical analysis is covered by the third and fourth chapters. The third chapter describes the main rapid methods of analyses of silicates, carbonates and phosphates in sufficient detail for the book to serve as a bench manual. The fourth chapter is a lengthy account of the chemical determination of minor elements in which the occurrence, detection and estimation of over fifty elements are described element by element.

The fifth to ninth chapters cover emission spectrography, flame photometry, X-ray spectrography and diffraction and fluorimetry. In each case the brief description covers basic principles, equipment and procedure. As an introduction for geologists with a

limited chemical background knowledge these chapters may serve some purpose. However, even in this context it would have been useful to outline the relative speed, precision and general applicability of each technique rather than cite limited, specific examples of their application. Some of the pitfalls of these techniques are understated, particularly matrix effects which are of prime importance in optical and X-ray spectrography and in X-ray diffractometry. A fuller description of matrix effects would have been of benefit to the beginner, particularly if ways of circumventing or overcoming such problems were outlined or, at least, referenced. There is, indeed, little reference to modern literature; nothing more recent than 1962 in relation to emission spectrography, or 1961 for X-ray spectrography! The latter is a technique in which enormous advances have been made in the last decade, for example the equipment shown in Figs. 70 and 71 is now essentially obsolete.

The final chapter, on chromatography, is more thorough and includes an outline of the basic principles, followed by detailed descriptions of paper and thin-layer chromatography, gas or vapour-phase chromatography and pyro-chromatography. The latter technique, developed by Lord Energlyn (W. D. Evans), includes an interesting account of geochemical applications.

In spite of the wide variety of techniques discussed in this book there are notable omissions, particularly important being atomic absorption spectrometry, which is rapidly gaining status as a major analytical technique in geochemistry. The lack of reference to modern work reduces the value of this book as an introductory text, since many physical methods of analysis have changed to such an extent that the methods and hardware of ten years ago are essentially redundant. Priced at £9.25, this book cannot be considered value for money.

D. M. HIRST

Maths and Music

The Acoustical Foundations of Music. By John Backus. Pp. xiv+312. (John Murray: London, March 1971.) £3.50.

MANY physicists have been interested in the physical principles of music, in how sounds and the particular quality of sounds are produced by musical instruments, in the admixture of sounds and the effect of the surroundings on what one hears, and in the many other problems connected with music, including the physiological questions about hearing and the voice. It is a fascinating subject for physicists who are interested in music or musicians who want to have some idea of "how things work".

This book is directed to musicians and particularly to those "with no scientific background and no mathematical equipment past the high school algebra they have already forgotten". The author goes on to say "thus they are representative of musicians generally". Is this so? One hopes not, but even if it is, twenty pages of elementary mechanics, including careful definitions of length, time and mass, seem rather unnecessary. This determination to keep mathematics to a minimum precludes the use of trigonometric functions and logs (although logs are explained in an appendix) and this leads to rather cumbersome explanations of several topics, for example, the various musical scales.

Given this limitation, the author describes the main features of the subject very well, but this has been done before by a number of authors. It is in the discussion and explanation of finer details that both musicians and physicists find most interest, because the fundamentals are in most cases fairly easy, but the exact description of what happens is often extremely complicated. Here the author is concerned to show, without too much complication, how some of these problems can be tackled and the results that have been obtained. He is also concerned to refute the "fallacies and superstitions that music could better do without". Most of these onslaughts seem to be fair comment but in the case of piano touch there is a point which seems not to have been made. The physicists' argument, strongly expressed by the author, is that when the hammer strikes the string it is "completely separate from the impelling mechanism attached to the key" and that "it follows that the pianist cannot independently control the quality of a single note on the piano by the manner in which he strikes the keys". The first statement is true, but the second does not follow because the stem carrying the hammer will bend and the bending is proportional to the acceleration while the final velocity depends on the integral of the acceleration. It is doubtful whether the experimental work done in the 1920s was of sufficient accuracy to resolve this point and it should be added to the very many unsolved problems which the author points to as requiring further investigation.

This constant reminder of how much work is waiting to be done is one of the very good features of this book, as is the treatment of wind instruments, so often neglected in books of this sort (the author plays the bassoon), and also the extensive bibliography, much of it of recent work, which will so much ease the path of those interested in the pursuit of some of these topics.

J. H. E. GRIFFITHS